

DeltaVision[®] Core
and personalDV
Restoration Microscopy
System User's Manual
Revision C
built with precisionware[®]

Legal Notices

Revision C of the *DeltaVision Core* and *personalDV* Restoration Microscopy System User's Manual.
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Other Reference Documents

User instructions for *DeltaVision Core* include the following publications:

Document	Purpose	Available for...
Online Help	Provides reference information for <i>softWoRx</i> and procedures that show how to use <i>softWoRx</i> tools	All <i>softWoRx</i> workstations
Product Notes	Provide examples and tips for using <i>softWoRx</i>	All <i>softWoRx</i> users (online at www.appliedprecision.com)
SoftWoRx Imaging Workstation User's Manual	Shows how to process, visualize, and analyze data.	All <i>softWoRx</i> workstations
Getting Started with QLM	Shows how to acquire photokinetic data with the QLM module	Acquisition workstations that have the optional QLM module

In addition to the above Applied Precision documentation, you will be provided with manufacturers' manuals for the microscope components. You may also be provided with manufacturers' manuals for cameras and other devices, depending on your system configuration. (Some manufacturers do not provide manuals for these devices.)

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Preface

This manual shows how to use the *DeltaVision* system to acquire images. It also shows how to maintain the system.

- *About This Manual* describes the information in each chapter.
- *Document Conventions* explains the typography, notes, and other conventions used in this manual.
- *Contacting Applied Precision, LLC* provides information about how to contact customer support.

About This Manual

This manual provides instructions for scientists who are using *DeltaVision* to acquire data. It also includes instructions and references for maintaining the system.

The *Introduction* provides a brief summary of the *DeltaVision* system features. Safety warnings and guidelines are provided in Chapter 2.

Chapters 3 - 6 show how to use the system to acquire data.

- Chapter 3, *Getting Started*, describes how to turn the system on, acquire an image, and run an experiment macro.
- Chapter 4, *Setting Up and Running Experiments*, shows how to set up experiment macros for 3D sectioning, Time lapse, Multiple wavelengths, Paneling (for stitching), and Point Visiting.
- Chapter 5, *Acquiring Data From Live Specimens*, provides information on how to use the DeltaVision Core system to collect images from live specimens.
- Chapter 6, *Data Collection Techniques*, describes how to determine the proper exposure time and provides guidelines for finding the areas of interest on a sample.

The remaining chapters provide information on how to maintain and configure the system.

- Chapter 7, *Facility Requirements and Components*, lists requirements and describes the *DeltaVision* system components.
- Chapter 8, *Changing Cameras and Filters*, describes how to replace cameras and how to install or replace filters.
- Chapter 9, *Maintenance*, shows how to change and align the xenon lamp, align the light path, clean the system, and change fuses.

The appendices include reference information and procedures for configuring the system.


Document Conventions

To make the information provided in this manual as easy as possible for you to locate and use, the following conventions are observed.

Lists


- Round bullets indicate options in procedures.
- 1. Numbered items are sequential steps for completing a procedure.
- Square bullets indicate items in a list.
- Arrows indicate single step procedures.

Notes, Warnings and Cautions


 **Note** Indicates information about the previous paragraph or step in a procedure.


 **Important** Indicates important or critical information about the previous paragraph or step in a procedure.

 **Tip** Indicates helpful advice.

 **WARNING:** Indicates important information regarding potential injury.

 **WARNING:** Indicates risk of explosion.

 **WARNING:** Indicates risk of shock.

 **CAUTION:** Indicates important information regarding potential damage to equipment or software.

User Interface Description Conventions

Boldface indicates the names of buttons, menus, dialog box options, and fields.

Initial Capitals indicate the names of windows, dialog boxes, and tabs.

ALL CAPITALS SAN SERIF indicates the name of a key on your keyboard or keypad, such as ENTER, DELETE, or STEP INCREASE.

Uniform width font indicates text to enter on a command line or in the GUI.

Contacting Applied Precision, LLC

If you have questions about *DeltaVision*, first refer to this manual or consult the online Help system. If you don't find the information you need, contact us at one of the following addresses.

Customer Service Hotline

Phone: 800-862-5166

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Introduction

This chapter provides an introduction to *DeltaVision*.

- *What is DeltaVision?* introduces the *DeltaVision* system and provides a history of its development.
- *What Can You Use DeltaVision for?* lists the supported imaging modes and summarizes the data acquisition options that are supported by *DeltaVision*.
- *What should you know to use DeltaVision?* summarizes the background and experience required to run the system.

What is DeltaVision?

The *DeltaVision* Restoration Microscopy System can be used to collect and analyze three-dimensional microscope images, acquired over long periods of time and on multiple samples. With the sophisticated *softWoRx* image analysis and model-building software, the system is a comprehensive package for biological image data collection, interpretation, and display.



History

The original restoration microscopes were designed and developed in the laboratories of Dr. John W. Sedat and Dr. David A. Agard at the University of California, San Francisco. Their first working system actively collected images as early as 1983. At that time, a *small* deconvolution (128x128x64) required overnight processing on a million dollar mainframe computer.

During the evolution of the UCSF microscope, it became clear that micropositioning was a critical part of the optical sectioning process. In particular, controlled movement of the focal plane relative to the specimen (the Z axis) was identified as a key to reliable deconvolution. To accomplish adequate Z scans, Dr. Sedat built a microscope stage using Applied Precision's Nanomotion™ micropositioning technology.

Before long, Dr. Sedat contacted Applied Precision cofounder Ron Seubert for detailed information about Nanomover performance. The relationship between UCSF and Applied Precision grew steadily. Later, in 1993, Applied Precision licensed the image restoration technology from UCSF and began development of *DeltaVision*. Collaboration between Applied Precision and UCSF still continues, for the benefit of both parties.

In October of 1993, Applied Precision shipped the first Applied Precision/UCSF hybrid to Michael Paddy at the University of Florida. In 1994, Applied Precision designed, built, and delivered a *DeltaVision* prototype to Paul Goodwin at the Fred Hutchinson Cancer Research Center, Seattle. In August 1994, the first commercial

DeltaVision microscope was shipped to Bethe Scalett at Lewis & Clark College, Portland, Oregon. All three of these systems are still active. The *DeltaVision* software has grown continuously since 1983, with contributions from scientific programmers, faculty, and graduate students at UCSF. Applied Precision's contribution to the software started in earnest in 1994.

The advances in computer and camera technology in the early 1990s resulted in the emergence of optical sectioning technology. For example, in 1993 Applied Precision's benchmark deconvolution ($512 \times 512 \times 64$) required 3 hours of processing time on a \$35,000 workstation. (The benchmark deconvolution is 16 times larger than the original deconvolutions performed in 1983.) Although only a few laboratories were able to afford \$35,000 for a computer, the lower cost warranted commercialization of a deconvolution microscope. In 1996, the same deconvolution required about 1/4 of an hour with a \$14,000 workstation, and 1/2 of an hour with an \$8000 machine. This thirty-fold increase in performance/price allowed a more widespread acceptance of deconvolution microscopy.

Advances in computing power and data storage have continued to benefit *DeltaVision* users. In 2003, a \$2500 workstation could perform the standard benchmark in less than four minutes, representing another twenty-fold increase in performance/price since 1996. The current workstations can now perform this benchmark in less than 30 seconds (under 20 seconds for *DeltaVision Core* and under 30 seconds for *personalDV*). Vast amounts of data generated by these experiments can be stored on local hard drives, by burning DVD's, or by transferring the data to other locations using high-speed network connections.

What Can You Use *DeltaVision* for?

DeltaVision Core and *personalDV* use research grade microscopes to collect optical images in one of the following imaging modes:

- Fluorescence
- Brightfield
- Phase contrast*
- Differential Interference Contrast (DIC)

* Phase contrast requires additional equipment that is available from Olympus distributors.

The following table summarizes the capabilities of the *DeltaVision Core* system.

Capability	Description
Digital Microscopy	Fluorescence Imaging. Also capable of Brightfield, Phase Contrast, and DIC imaging.
Automated Optical Sectioning, Time-lapse, Point visiting	Optical sectioning, filter changes, and shutters are coordinated by the controller.
Quantitative Processing	Image processing and 3-D reconstructions of multi-dimensional data files.
Image Display and Analysis	3-D reconstructions can be visualized, rotated, and enhanced.

DeltaVision supports a wide array of imaging applications, including: Cytoskeletal Studies, RNAi experiments, Live Cell Imaging, Cell Cycling Studies, Protein Translocation, and Protein Pathway Analysis.

Standard Data Collection Options

DeltaVision supports the following types of data acquisition:

3D Imaging

To acquire “3D data,” you can set up *DeltaVision* to acquire a series of images along the Z axis. The *softWoRx* workstation provides a sleek interface that allows you to control the optical sectioning through a specimen. Behind the scenes, macro language provides automated computer control of sample position, optical filters, and shutters. After image data acquisition, a series of image processing algorithms improve image resolution. Three-dimensional information can be reconstructed and then visualized in a variety of ways that allow quantitative measurement and analysis.

Multiple Filters

You can acquire images through several filters and combine them into one image file.

Panel Collection

Panel collection acquires a series of images with adjacent fields of view. You can stitch these images together to create images that are much larger than a single field of view. This is especially useful when you want to collect data at a high magnification over a large area.

Time-Lapse

You can run macros to acquire time-lapse images and use the data to create time-lapse movies. This is especially useful for studies of live samples or for experiments that use lasers.

Point Visiting

Point Visiting allows you to acquire data from several areas of interest during a single experiment. You can select which points to monitor on your sample and save them in a list that contains the exact stage coordinates of each point. When you run the experiment, *DeltaVision* reads the coordinates for the points in the list, moves the stage to each point, and captures an image. This process is repeated at specified time-lapse intervals. For live specimens, this significantly improves the lab efficiency of experiments by allowing you to monitor multiple points of interest in a single session.

Autofocus

You can use Autofocus to automatically focus when you are viewing a sample or when you are running an experiment.

Köhler and Critical Illumination

You can easily switch between Critical and Köhler Illumination. Köhler Illumination provides very even specimen illumination across the field of view. You will typically use Köhler Illumination for most of your data collection. Critical Illumination directs the entire light source to the size of the detected area. It is useful for low abundant probes that require more light.

Live Cell Imaging

You can set up a controlled environment to acquire data from live specimens. *DeltaVision* supports an optional environmental chamber that you can use to control temperature and inject carbon dioxide.


DeltaVision also includes software that is specially designed to acquire data from live specimens:

- Cell tracking is used for point visiting time-lapse experiments. It automatically changes the coordinates of a point to follow a cell as it moves.
- Real-Time Z Sweep Acquisition (or Optical Axis Projection) allows you to quickly acquire 2D projections of specimens.
- Real-Time Deconvolution provides previews of deconvolved images as they are acquired.

- Reference Imaging is useful for acquiring reference images that can be used for Differential Interference Contrast (DIC) and other techniques.


Laser Photo-bleaching and Photo-activation

If your system has the Quantifiable Laser Module (QLM) hardware module, you can use *DeltaVision* to run and analyze laser photo-bleaching and photo-activation experiments. The QLM supports up to three lasers and provides software to control and to analyze the data that is obtained from these experiments.

 **Note** The QLM hardware is not an available option for *personalDV*.

TIRF (Total Internal Reflection Fluorescence)

If your system has the TIRF hardware module, you can use *DeltaVision* to run and analyze TIRF experiments. TIRF is used in a number of research applications, such as cellular protein and vesicle trafficking, focal cellular adhesions, single biomolecule dynamics, studies in neuroscience, and cell-to-cell communications. The ability to excite molecules on the surface of a specimen while eliminating the fluorescence from the depth of the sample makes TIRF a valuable tool for examining cell surface structure and protein dynamics.

 **Note** The TIRF hardware module is not an available option for *personalDV*.

What should you know to use *DeltaVision*?

This document assumes that you are familiar with the basics of microscopy. Correct operation of the microscope is fundamental to obtaining quality images with *DeltaVision*. In addition, an understanding of image processing basics will help you use the system to its full potential. To manage the computer systems, some familiarity with Linux workstations and IBM-type personal computers is helpful.

We have taken care to ensure that *DeltaVision* is straightforward to use, reliable, and complete. Please report errors and problems with *DeltaVision* to Applied Precision using the e-mail address servicehotline@api.com, or alternatively, use the problem report form in *Appendix D: Reference Information*.

2

Safety

The precautions detailed in this chapter must be carefully observed to prevent possible personal danger:

- *UV Exposure* discusses potential for UV exposure from the xenon arc lamp.
- *Bright Light Exposure* warns about bright light exposure from the transmitted light source installed in the microscope.
- *Burn* provides guidelines for avoiding burns from the xenon arc lamp. (The arc lamp reaches very high temperatures.)
- *Shock* includes warnings about potential shock hazards. (Hazardous voltages are present even when the system is disconnected from the AC main power outlet.)
- *Damage Prevention* describes actions that can damage the system.
- *Warning Labels* describes the system labels.

Additional safety guidelines for maintenance and alignment are detailed in *Chapter 9: Maintenance*.

UV Exposure

Since the xenon arc lamp emits ultraviolet (UV) light, there is a danger of exposing your eyes and skin. Loss of eyesight could occur if unfiltered light from the xenon arc lamp reaches your eyes.

To prevent UV exposure:

- Open the shutter only when an excitation filter is engaged.
- Do not open the xenon arc lamp housing during operation. See *Chapter 9, Maintenance* for detailed instructions on changing the xenon arc lamp bulb.

Bright Light Exposure

While the transmitted light source installed in the microscope does not present possible UV exposure, it could cause discomfort under certain conditions.

- You must be aware of the eyepiece filter wheel when viewing a specimen through the microscope oculars. Make sure that the proper filters are in place so that your eyes are not suddenly exposed to a bright flash of light when the transmitted light shutter is opened.
- Do not look through the eyepiece while switching filters. Your eyes can be exposed to unfiltered light during the filter transition.

Burn

The xenon arc lamp reaches a very high temperature when lit. Never touch the housing during operation. Never remove the housing during operation or before allowing it to cool completely. Carefully follow the directions found in *Chapter 9, Maintenance*, for changing the lamp.

Shock

Hazardous voltages are present even when the system is disconnected from the AC main power outlet.

To replace the transmitted light source LED, follow the instructions in the manuals that are included with your microscope. To replace the xenon arc lamp, follow the instructions found in *Chapter 9: Maintenance*. No other system components contain user-serviceable parts and do not warrant disassembly.

If the High Res Camera coolant fluid is leaking, shut down the system and contact Applied Precision Customer Service. (See *Customer Service Hotline* on page xii.)

Damage Prevention

The following actions could damage the system:

- Moving the stage to the home position with the objective up could break or scratch the objective. The stage could be driven into the objective and potentially scratch the top lens or compress the lens housing, causing a leak, crack, or lens misalignment.
- Disconnecting cables before the system is completely shut down will damage one or more of the electronic circuit boards.
- Disconnecting cables to the camera when the power is on can damage the camera.
- Touching optical filters or the polychroic beam splitter contaminates them with oil and can lead to premature failure or poor image quality. For cleaning information, see *Chapter 9: Maintenance*.
- Bending the fiber optic cable into a coil with a diameter less than 24" will damage the cable.
- Using improperly rated replacement fuses can create a fire hazard and may result in damage to components. Use only the fuse types listed on the component or in the manual.
- Leaving a camera out of the tray when it is not in use presents an opportunity for the camera to fall to the floor and break. Always place the camera in the camera tray when it is not in use.

Warning Labels

Warning labels have been applied to the components of the system that pose a potential hazard to the user. The labels have been duplicated here and carefully explained. Please read this section carefully.

 **Note** For a description of DeltaVision components, see Chapter 7, Facility Requirements and Components.

DeltaVision Warning Labels



Hazardous Voltage Warning Label

This label indicates the danger of electric shock. This label is found on the xenon arc lamp housing.



Caution or Warning Label

This label indicates a danger of personal injury or possible damage to equipment. It is accompanied by an explanation of the specific danger. This label may be found on the microscope, the High Res camera, the Fast Camera, or the workstation.

3

Getting Started

This chapter shows how to get started with *DeltaVision*.

- *Before you start* includes a checklist of things that you need to have before you can acquire images for your sample.
- *Getting Familiar with DeltaVision* describes the key controls that you will use to direct the light path, focus, and select filters. It also introduces the keypad and joystick.
- *Turning DeltaVision on* shows how to turn on the system.
- *Acquiring an Image* shows how to place the slide and the objective, find the sample, and acquire an image.
- *Saving Image Data* describes how to set up a personal data folder and how to save an image file.
- *Turning DeltaVision Off* shows how to turn off the system.

Before you start

Before you start, make sure that you:

- Select the proper oil for your objective. The immersion oil kit includes 18 oils with refractive indexes that range from 1.500 to 1.534, in increments of 0.002. (For personalDV, the kit includes 6 oils that range from 1.512 to 1.522.) To calculate the best refractive index for your application, follow the instructions in *Appendix A: The Immersion Oil Kit* on Page 155. If you are working at standard temperature and pressure, the oil with a 1.516 refractive index is generally a good place to start. For work at 37 degrees C, use the oil with a 1.520 refractive index.
- Know your login ID and password.
- Prepare your sample using the recommended practices that are documented in the following references. (If you are untrained in sample preparation, consider attending a course like the one shown below.)

Books on Cell Biology Methods

Current Protocols in Cell Biology. Bonifacino, Dasso, Lippincott-Schwartz, Harford, Yamada ed. Wiley Press, <http://www.wiley.com/legacy/cp/cpcb/>

Cells, A Laboratory Manual. Spector, Goldman, Leinwand ed. Cold Spring Harbor Press, 1998.

Video Microscopy: The Fundamentals (2nd Edition). Inoue and Spring, Plenum Press, 1997.

Digital Microscopy (3rd Edition), Methods in Cell Biology Vol. 81. Sluder and Wolf, Academic Press, 2007.

Papers on Sample Preparation

Rines DR, He X, Sorger PK. Quantitative microscopy of green fluorescent protein-labeled yeast. *Methods Enzymol.* 2002;351:16-34.

Hutchins JR, Moore WJ, Hood FE, Wilson JS, Andrews PD, Swedlow JR, Clarke PR. Phosphorylation regulates the dynamic interaction of RCC1 with chromosomes during mitosis. *Curr Biol.* 2004 Jun 22;14(12):1099-104.

Courses in Microscopy and Cellular Imaging

In Situ Hybridization, Immunocytochemistry, and Live-cell Imaging. Dernberg, Hu, and Murray Course Directors, Cold Spring Harbor, October (annual).

Analytical and Quantitative Light Microscopy, Sluder and Wolf Course Directors,
Marine Biological Laboratory, Woods Hole, MA, May (annual).

Getting Familiar with *DeltaVision*

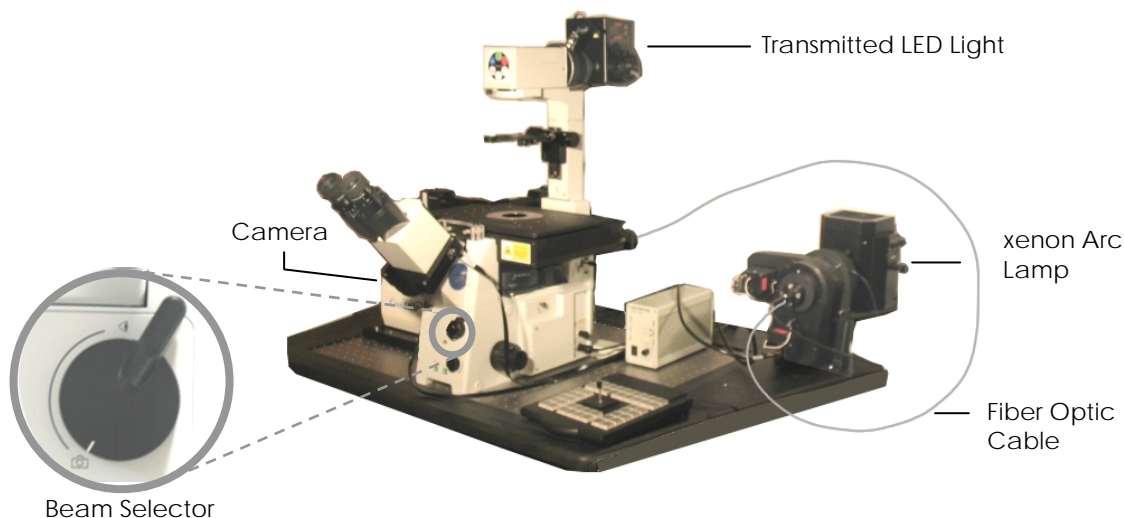
Before you acquire an image, become familiar with the key *DeltaVision* controls for:

- Controlling the Light Path
- Focusing
- Choosing Filters
- Using the Keypad and Joystick

Most of the manual controls for controlling the light path, focusing, and choosing filters are similar to those that you will find on any microscope. Additional controls for moving the stage and acquiring images are provided by the keypad and joystick.

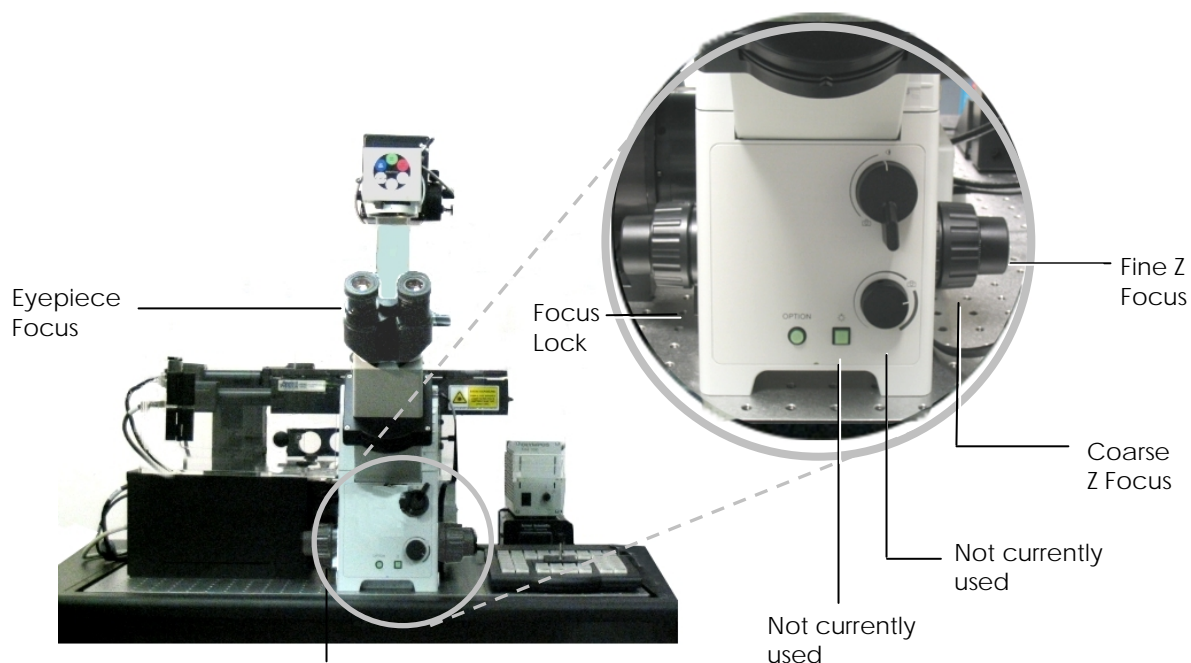
Controlling the Light path

DeltaVision provides a transmitted LED light and a xenon arc lamp. The transmitted light works the same as the light source for a traditional microscope, with the light path directed on the specimen from above. The xenon arc lamp provides excitation light directed through the back of the microscope and focused on the specimen from below. You can use the Beam Selector to direct either light path from the specimen to the eyepiece or to the camera.



Focusing

There are three manual focus controls and a Focus Lock on the microscope. These controls are similar to those on other systems.



Eyepiece Focus Use the Eyepiece Focus (on the left ocular) to focus the eyepiece.

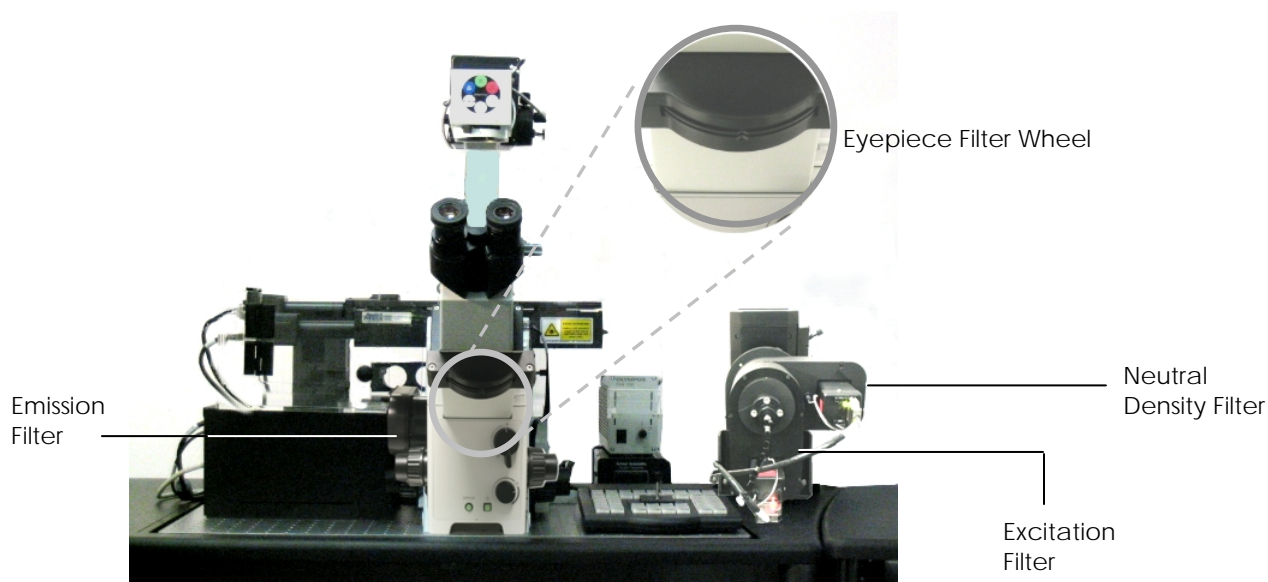
Focus Lock Use the Focus Lock to lock or unlock the Z focus.

Fine Z Focus Use the Fine Z Focus knob to move the objective in very small increments. It is used to focus on the focal plane.

Coarse Z Focus Use the Coarse Z Focus knob to move the objective in large increments. It is typically used to lower the objective when the system is initialized or to move the objective up to the slide until the oil is touching the slide.

Choosing Filters

Choosing and controlling filters is a key for any fluorescent probe experiment. When a fluorescent probe is excited by a specific wavelength, it emits light at another wavelength. Choosing the right filters for the dyes in your sample allows you to obtain a complete set of data specifically from your probe without interference from other wavelengths.



DeltaVision provides five different types of filters for controlling the fluorescent light path:

- **Neutral Density** filters reduce the amount of light that illuminates your sample when you are using fluorescence. *DeltaVision* provides six filters that block from 0 to 99.9% of all light.
- **Excitation** filters block all but one band of wavelengths to provide a specified range of light to excite the fluorescent probes in the sample.
- **Polychromatic Beam Splitter** reflects the excitation wavelengths to the sample and transmits the emission wavelengths from the sample. *DeltaVision* ships with a standard polychromatic beam splitter for DAPI, FITC, TRITC, and Cy5. Other beam splitters ship with optional live-cell sets.
- **Emission** filters allow only a single band of light from the excited probe to reach the camera.
- **Eyepiece** filters are emission filters that allow only a single band of light from the excited probe to reach the eyepiece and your eyes.

These filters are arranged in sets that are associated with specific dyes. (For example, a dye such as DAPI is typically used with a DAPI Excitation filter, a DAPI Emission Filter, a DAPI Eyepiece Filter, and a 100% Neutral Density filter.)

You can choose filter sets manually by rotating the eyepiece filter wheel. The filter sets are synchronized so that when you change an eyepiece filter, the neutral density filter, excitation filter, and emission filter automatically change.

Using the Keypad and Joystick

The keypad and joystick are used to move the stage, open shutters, acquire images, and control other acquisition options. Key controls are shown below.



Key controls on the Keypad

Acquire Image

Acquires an image and displays it on the monitor. Use this key when you are scanning through your sample and using the eyepiece to find a region of interest, or when you want to get a quick look at the specimen on the monitor.

Ex Shutter

Opens or closes the Excitation (i.e., Fluorescence) shutter. You will use this control frequently to open and close the shutter. Because the shutter is designed to protect your eyes from exposure to ultraviolet light, it automatically closes each time that a filter wheel is moved. It must be reopened with the EX SHUTTER button.

Trans LED Source

Toggles the transmitted light LED between off and on. (Subsequent to changing the transmitted light source from halogen to LED for *DeltaVision Core* and *personalDV*, an actual shutter is no longer necessary.)

Slow, Medium, and Fast

Control the speed that the stage is moved by the joystick or keypad arrows. It's usually best to start with medium.

The Joystick

Controls stage movement. Use the joystick to move the stage in the direction that you point with the joystick (for example, moving the joystick up moves the stage away from you, moving joystick left moves the stage to the left, and so on).

Turning *DeltaVision* On

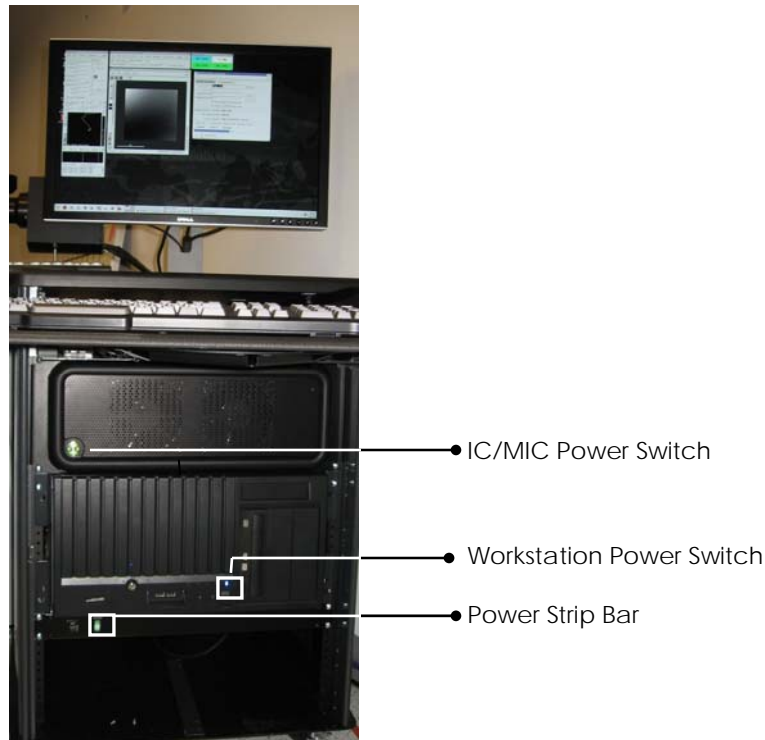
Use the following instructions to turn the system on for day-to-day use. For instructions that show how to turn on *DeltaVision* after a system shutdown, see *Shutting Down and Starting the System* on Page 133.

To turn on *DeltaVision*:

1. Turn on the power strip bar.

Note For *personalDV*, there is no power strip bar. Begin the power-up process with Step 2.

2. Turn on the IC/MIC.



The Main Power Switches in the *DeltaVision* Cabinet (acrylic cover not shown)

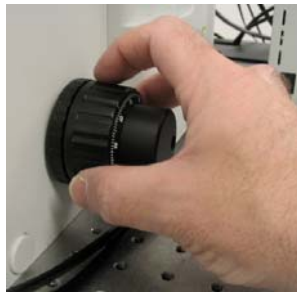
3. If the monitor is off, turn it on.
4. If the Workstation is off, turn it on and wait for it to boot up.
5. Log on to the Workstation.
6. Remove any slides from the stage.
7. On the desktop, double-click the DV icon to open *softWoRx*.
8. On the *softWoRx* menu, choose **File | Acquire (Resolve3D)** and follow the prompts, allowing the system to initialize.



9. Release the Focus Lock by turning it clockwise (when facing the lock) until it is loose.



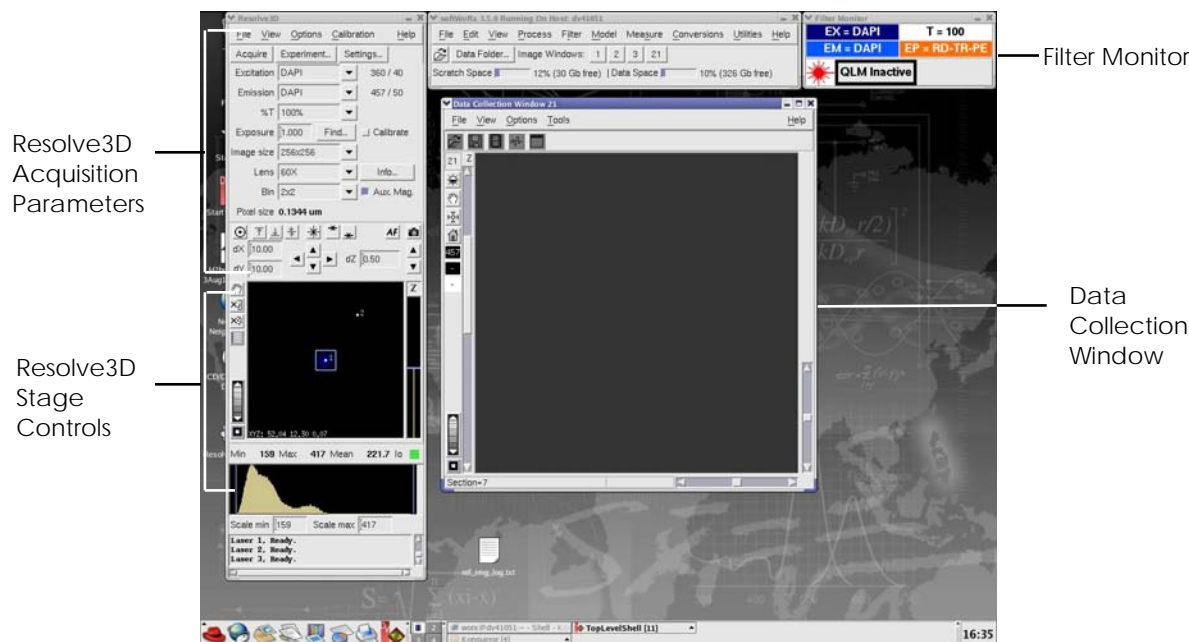
10. Lower the objective by turning the Coarse Z Focus knob away from you (clockwise) when facing the knob.



CAUTION: Always lower the objective before you initialize the system to prevent damage to the objective lens.

11. A dialog box is displayed prompting you to lower the objective before continuing. After you lower the objective, select **Initialize** to initialize the system.

The Resolve3D window, the Data Collection Window, and the Filter Monitor dialog box open on the desktop.



The Resolve3D window includes acquisition parameters and controls for moving the stage. The Data Collection window displays images as they are acquired and the Filter Monitor displays the filters that are selected.

Note You will use the Resolve3D window throughout the data collection process. For more about this window, see *The Resolve3D Window* on Page 184.

Acquiring and Saving Data

To acquire an image of your sample, you will need to:

- Set up *DeltaVision* by placing the slide and selecting the appropriate filters.
- Find the sample so that it is visible in the eyepieces.
- Acquire an image with the camera.

Setting Up the Sample for Image Acquisition

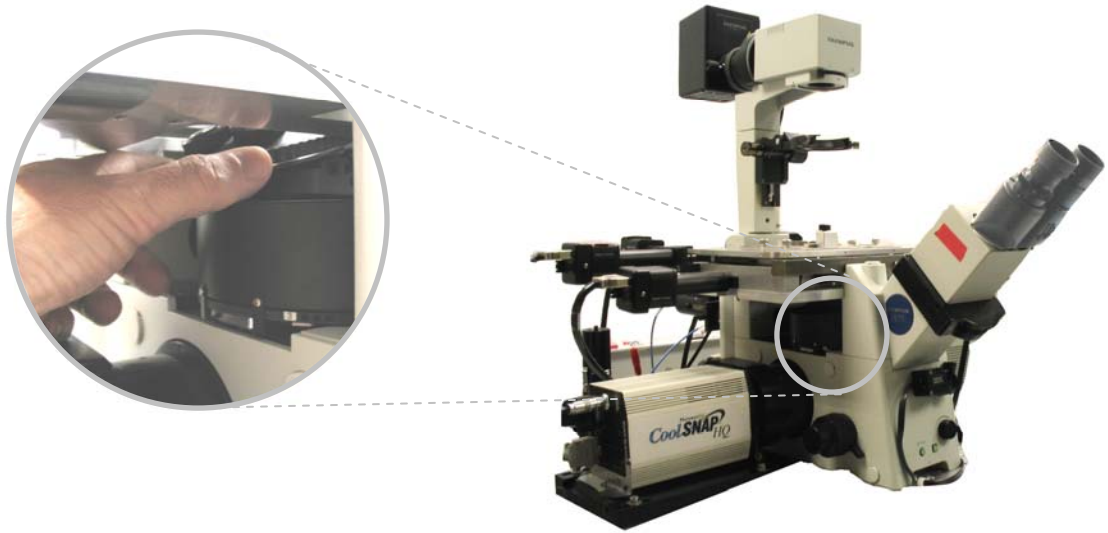
Setting up *DeltaVision* for imaging includes placing the sample on the stage so that it is in contact with the immersion oil and selecting the appropriate filter set for the fluorescent probe use to label your sample.

To prepare for image acquisition:



1. Rotate the Coarse Focus knob so that the top of the knob rotates away from you (while you are sitting in front of the microscope) to move the objective all the way down.

2. Rotate the objective turret to select an objective.

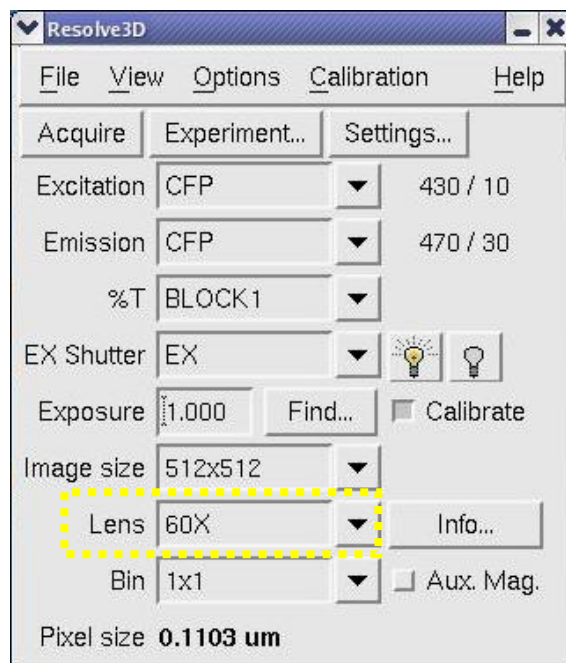


Use the objective turret to select an objective



Tip Use a high power objective (e.g., 60X) to find the sample. The approach for finding a sample with a fluorescent microscope is different than what you would use for a transmitted light microscope. With transmitted light, a typical approach is to find the sample with low powered objectives and then increase power after you find the sample. But with a fluorescent light, the sample is often not visible at low powers. The best approach for finding a sample with fluorescent light is to start with a high power objective.

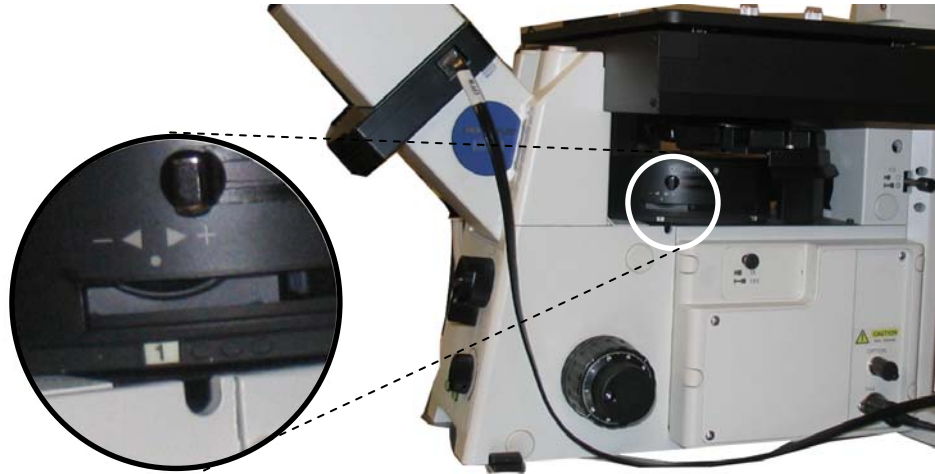
3. In the Resolve3D Lens list, select the same objective.





CAUTION: Make sure that the objective is selected in the Resolve3D Lens list.

4. Rotate the dichroic filter wheel to select the appropriate dichroic mirror. For standard filters, use position 1.



5. In the Resolve3D Window, click the Info button to open the Lens Information window.

File		Lens		Help		
Lens ID		10105		Name		
Manufacturer		Olympus		Olympus 10X/0.40, D Plan Apo UV, phase, fluor free, IM		
Manufacturer P/N		1-LP331		APLLC P/N		
				Unknown		
Magnification		10.0		NA		
				0.40		
Working Distance (um)		3100.0		Focal Length (um)		
				15690.0		
Refractive Index	Standard Refractive Index	1.000		Recommended Refractive Index		
				1.000		
Optical Conditions						
Optical Conditions	Distance From Coverslip to Specimen (um):		0.0		Coverslip Thickness (um):	
					170.0	
	Temperature (C):		25.0			
Specimen Refractive Index:		1.470		Glycerol - 1.470		
Resolution Calculations						
Resolution Ratio (Z/XY):		9.2		Depth of Field (um):		
				3.16		
Maximum XY Pixel Size (um):		0.48		Recommended Z Step Size (um):		
				4.35		

6. In the Optical Conditions fields, enter the conditions for the sample.

7. Note the displayed value in the Recommended Refractive Index field and use an oil with that refractive index.



CAUTION: Before you start each data collection session, calculate the oil. Your oil selection should be the same if the sample and conditions are the same.

8. Place a drop of oil on the objective. Be sure to use the proper oil (see Page 12).



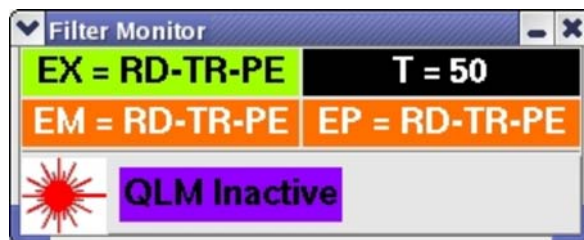
CAUTION: Do not touch the glass dropper to the objective.

9. Place a drop of oil on the coverslip. Be sure to use the proper oil (see Page 12).
10. Mount the slide on the Repeatable Slide Holder with the coverslip down.
11. Use the Adjustment knob on the Repeatable Slide Holder and the joystick on the Keypad to center the coverslip over the objective.



Tip If you have the Repeatable Slide Holder, you can record the position of your slide. This is useful when you are performing a Point Visiting experiment and you need to remove the slide before you are finished (see Page 100).

12. Rotate the Coarse Focus knob toward you to move the objective up until the objective is just in contact with the oil. From this point on, use only the fine focus knob to raise and lower the objective.
13. Rotate the eyepiece filter wheel (below the oculars on the scope) to select the filter for the probe that you used to stain your sample. If your sample has more than one probe, select the one with the brightest fluorescence (typically DAPI). The selected filter is displayed on the Filter Monitor window.



The filter names are displayed in the Filter Monitor dialog on the right hand side of the workstation screen. As you rotate the filter wheel, the filter name next to **EP** (eyepiece) changes and the **EM** and **EX** (emission and excitation) filters change automatically to match.

The displayed colors match the wavelength of the filters. If a QLM module is installed (not available on *personalDV*), the Filter Monitor also displays the wavelengths of the lasers that are available on your system.



Note You must move the filter wheel to initialize the Filter Monitor.

Standard Filter Set

Appropriate Probes	Filter Name	Excitation	Emission
DAPI, Hoechst, Coumarin	DAPI	UV, 350nm	Blue, 455nm
Fluorescein, GFP, CY2, Al488	FITC	Blue Green, 490nm	Green, 525nm
Rhodamine, Texas Red, Cy3,	TRITC	Green, 555nm	Orange, 605nm
CY-5®	CY-5	Red, 645nm	Infrared, 705nm

Live-cell Filter Set


Appropriate Probes	Filter Name	Excitation	Emission
CFP	CFP (optional)	Deep Blue, 430 nm	Blue, 470nm
YFP	YFP (optional)	Blue Green, 500 nm	Yellow Green, 535nm
mCherry	mCherry (optional)	Yellow, 572nm	Red, 632nm
EGFP	EGFP (optional)	Blue, 470nm	Green, 525nm

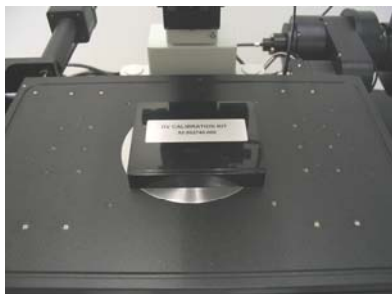
See Optical Filters on Page 93 for more information.

Finding the Sample

You will need to find the sample in the eyepieces and then focus on the sample to set the focal plane for imaging.


To find the sample:

1. With the sample on the stage and the filters selected as shown in the previous procedure, set the Beam Selector to **Eye**. 
2. Turn off the lights in the lab. If you cannot turn off the lights, place a box over the sample to reduce the amount of ambient light.



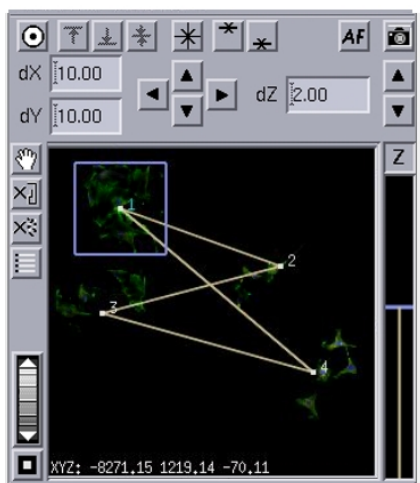
3. Open the Excitation shutter by pressing the EX SHUTTER button in the lower left corner of the keypad.

You should see light through the objective. The light on the stage should be the same color as the Excitation filter that you selected. (For example, if you selected DAPI, the light should be very deep violet. It may be hard to see.) Be sure the eyepiece filter position matches the desired excitation filter position.

 **Note** The Excitation shutter is designed to protect your eyes. Each time a filter wheel is moved, the shutter automatically closes and must be reopened with the EX SHUTTER button.

4. Focus to find the focal plane. Turn the Fine Focus knob toward you to slowly raise the objective until you see a cloud of emission color. Then use the joystick to move the stage. If the cloud moves, you have found the sample. If not, you may be seeing colors from lens effects. Continue to focus until the image is sharp and clear.
5. Use the joystick to move the stage around. Change the speed of the movement with the SLOW, MEDIUM, and FAST keys on the Keypad. When you find a sample, place it in the middle of the field of view.

On the stage View, note the stage trails that show where you have moved the stage in XY.



Acquiring an Image


To acquire a *DeltaVision* image, you'll need to direct light to the camera and work with the images that are displayed in the Data Collection window until you are satisfied. Then you can save the image as a *DeltaVision* file or create and run experiments, as shown in *Creating and Running an Experiment Macro* on Page 31.

To acquire an image:

1. On the keypad, press EX Shutter to close the shutter.
2. Switch the beam selector to camera.
3. In the Resolve3D window **Exposure** field, enter an exposure time (in seconds). A good starting exposure time is 0.1 second (see *Finding Exposure Time* on Page 78).



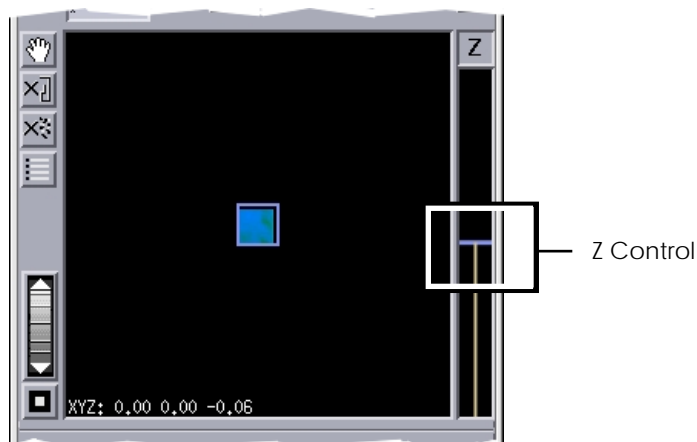
Tip You can also click the **Find** button on the Resolve3D window to find a good exposure time. Use **Find** carefully. This option can photo-bleach the specimen. You need to be particularly careful when determining exposure times for live cells. When using **Find** for live cells, start with lower exposure times by changing the Target Intensity Value to around 200 counts.


4. Click  to acquire an image.



Tip You can also use Auto Focus to focus the sample as follows: On the Resolve3D window, click **AF** to auto focus the sample. (If your image is far out of focus, you may need to click **AF** more than once.)

5. To focus, slide the Stage Z Control bar up or down.

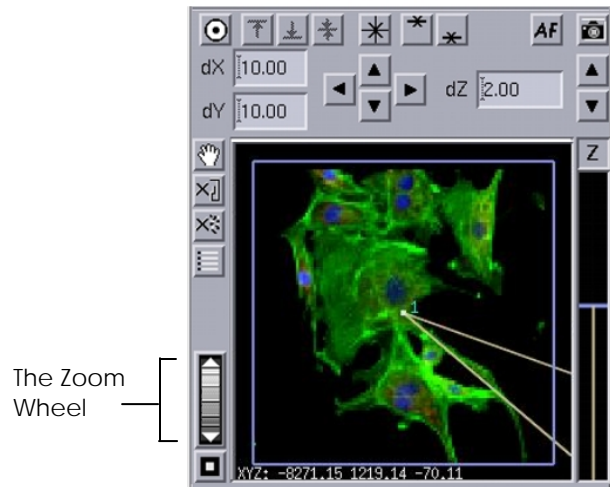


6. To center the image, click  and then click on the object that you want to center in the Image window.




Tip You can also acquire an image by clicking the ACQUIRE IMAGE button, right clicking on the stage view, or choosing **File | Acquire** on the Resolve3D menu.

- To enlarge the thumbnail image that appears in the stage view, drag the mouse down over the zoom wheel.



Note Thumbnails appear only when the Show Stage Thumbnails option is selected on the Misc tab on the Resolve3D Settings window.

- To clear the thumbnail image, click .

Saving Image Data

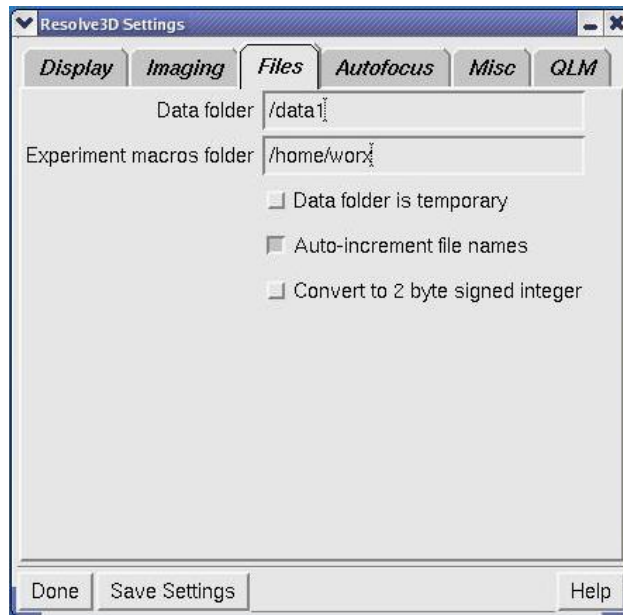
After you acquire an image, use these instructions to set up a personal data folder and use the Snapshot utility to acquire a single image.

Setting up a Personal Data Folder

You can set up a personal data folder in which to save your images. After you create and save this folder, *DeltaVision* automatically uses it as the data folder when you log on to the system.

To set up a personal data folder:

- At the top of the Resolve3D window, click the **Settings** button to open the **Resolve3D Settings** window. Then click the Files tab.



2. In the **Data folder** field, enter the directory (e.g., /data1/myData) in which you want to save your images. To create a new folder, type the name of the folder after /data1 (e.g., /data1/myNewData) and select **Save Settings**.

! **Important** Store all files in the /data1 directory unless you are instructed otherwise by your system administrator.

💡 **Tip** If you are using an existing folder for your Data folder, you can drag and drop the folder from the Linux File Manager to the Data folder field.

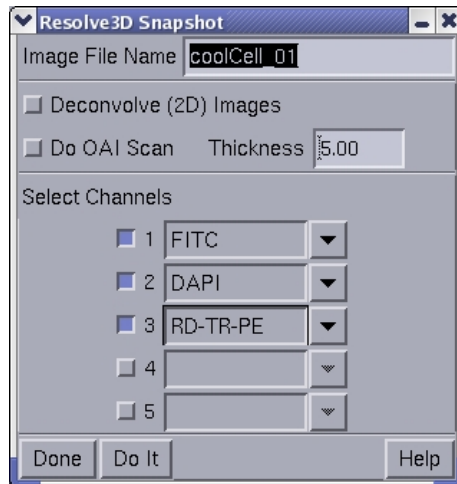
3. To reset the default Data folder when you log out, select the **Data folder is temporary** option.

Saving a Single, Multi-Channel Image

Sometimes it is desirable to acquire a single, multi-channel image. The Snapshot utility helps you quickly create a 2-D, multi-wavelength image without having to run a full experiment.

To acquire a single Multi-channel image:

1. Choose **File | Snapshot** from the Resolve3D window to open the Snapshot dialog.



Tip You can also open the Snapshot dialog by right clicking on the Resolve3D window to open a shortcut menu and choosing **Snapshot**.

2. In the **Image File Name** field, enter a file name. Then select which channels to save. (Snapshot uses the exposure conditions that are displayed in the Resolve3D window for those channels.)



Note *softWoRx* adds the _R3D.dv extension to the file name. If you enter a file name without a directory path, the file will be located in the current data folder. You can specify to place the file in another directory by including the path in the file name (e.g., /tmp/myfile).



3. Click **Do It**.

The Image window opens and displays the new image. The image is saved as a *DeltaVision* file that you can open in *softWoRx*.

Turning *DeltaVision* Off

Use the following instructions to turn off *DeltaVision* on a daily basis. For instructions that show how to shut down the system, see *Shutting Down and Starting the System* on Page 133.

To turn off *DeltaVision*:

1. Turn off the xenon light source using the bulb  icon on the Resolve3D main menu. Clicking the icon will switch it to the off  state.
2. Save all data on the workstation.
3. On the *softWoRx* menu bar, select **File | Exit**. Then exit all other workstation applications.

4. Log out of the workstation account.
5. Press the IC/MIC power switch to turn off the IC/MIC. Wait 30 – 60 seconds for the IC/MIC to power down.

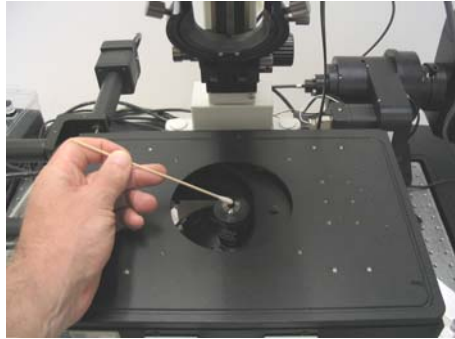


Note If the IC/MIC fails to turn off within 30 – 60 seconds after pressing the power switch, press and hold the IC/MIC power switch for 5 seconds to turn off the device.

6. Clean the objective with a clean, unused cotton swab or lens paper to remove all of the oil on the objective. Then apply chloroform to a cotton swab, gently roll it over the objective lens once, and discard it.



CAUTION: After you finish imaging, Always clean the objective with a clean, unused swab or lens paper. Never reuse swabs or lens paper.



7. Lower the objective by turning the Coarse Focus knob.

4

Setting up and Running Experiments

A *DeltaVision* experiment is a macro that runs a set of commands to collect image data. You will use experiments to acquire almost all of your data. This chapter shows how to set up and run experiments that use several *DeltaVision* data collection options. It includes:

- *Creating and Running an Experiment Macro*
- *Sectioning Specimens for 3D Images*
- *Selecting Filters*
- *Point Visiting*
- *Collecting Panel Images Over Large Areas*
- *Using the Multiplexed Wavelength Option*

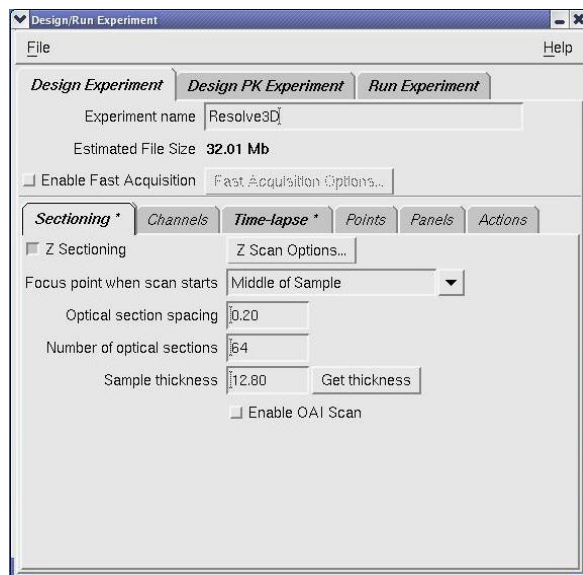
Creating and Running an Experiment Macro

DeltaVision Experiment macros are files that include settings and commands for acquiring data with *DeltaVision*. Macros allow you to automate data collection. After you use the *DeltaVision* interface to create a macro, you can run it to automatically collect data. Macros can be very simple or they can be very complex

and include numerous options. After you create a macro, you can use it as a template to modify and create other macros.

To create and run an experiment macro:

1. At the top of the Resolve3D window, click the **Experiment** button to open the **Design/Run Experiment** window.
2. Click the Design Experiment tab.



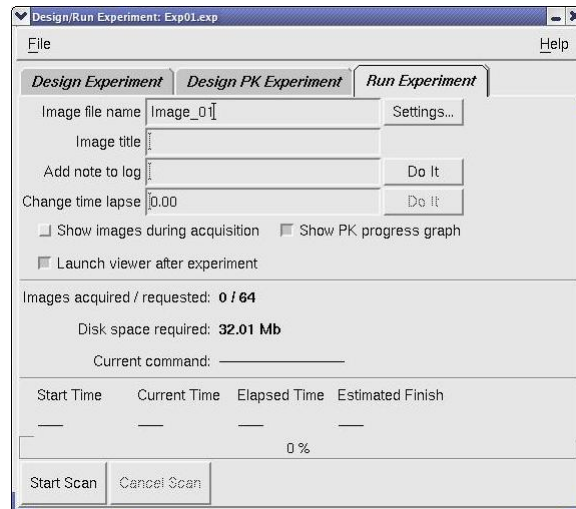
3. In the **Experiment Name** field, enter a name for the macro.



Note By default, SoftWoRx uses Resolve3D as the experiment name. This is a special disposable name that does not require confirmation to overwrite. If you do not intend to reuse an experiment, using Resolve3D for the experiment name is the simplest method.

Refer to *Sectioning Specimens for 3D Images*, immediately following this section for the specific components of designing an experiment.

4. From the **File** menu, choose **Save**. The macro name appears in the Design/Run Experiment window title bar.
5. Click the **Run Experiment** tab.



6. In the **Image file name** field, enter the file name.



Notes

#1 Do not use file names that include spaces or special characters (e.g., %, /, \, ", !, \$, #, ~, *, &, @, ', +, =).

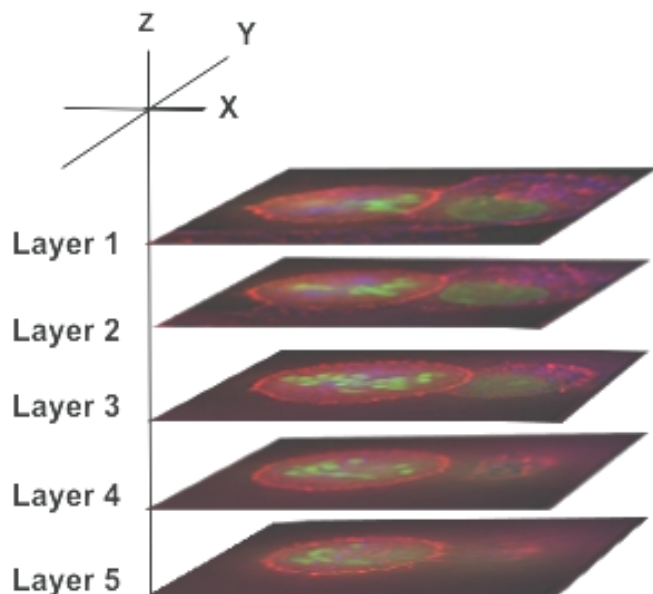
#2 The image file name has an _R3D.dv extension. This is the unprocessed (i.e., raw) Resolve3D image. A file with a .log extension is also created. The .log file is a text file containing information about the image file, including the macro and objective that were used to collect the image.

7. Click **Start Scan**.

The macro collects the image and saves it in your data folder (see *Setting up a Personal Data Folder* on Page 26). To process the image, use the deconvolution module in the *softWoRx* program, as shown in the *softWoRx Imaging Workstation User's Manual*.

Sectioning Specimens for 3D Images

You can acquire images of multiple sections. This data can be used to create 3D projections, view cross sections, and create volumetric and line models.



When you set up a Z scan on *DeltaVision*, you normally begin by defining a focus range to use. This is accomplished by marking the top and bottom of the sample. You then focus on a plane of interest. The focus range is taken above and below the plane of interest.



If you are new to 3D microscopy, you may have never had to deal with the specimen's three-dimensional size (except in relation to the rest of the specimen). Manually operated microscopes provide very little information about real distances in Z, and even X and Y. Once you start viewing your specimens in three-dimensions, however, you will become accustomed to the relationship between the microscope stage, sample, and optics, and will find that designing a Z scan is usually straightforward.

Use the following procedure to create and run a 3D experiment. To determine the Focus point when scan starts, Optical section spacing, Number of optical sections, and Sample thickness refer to *Guidelines for Designing and Running 3D Experiments* on Page 37.

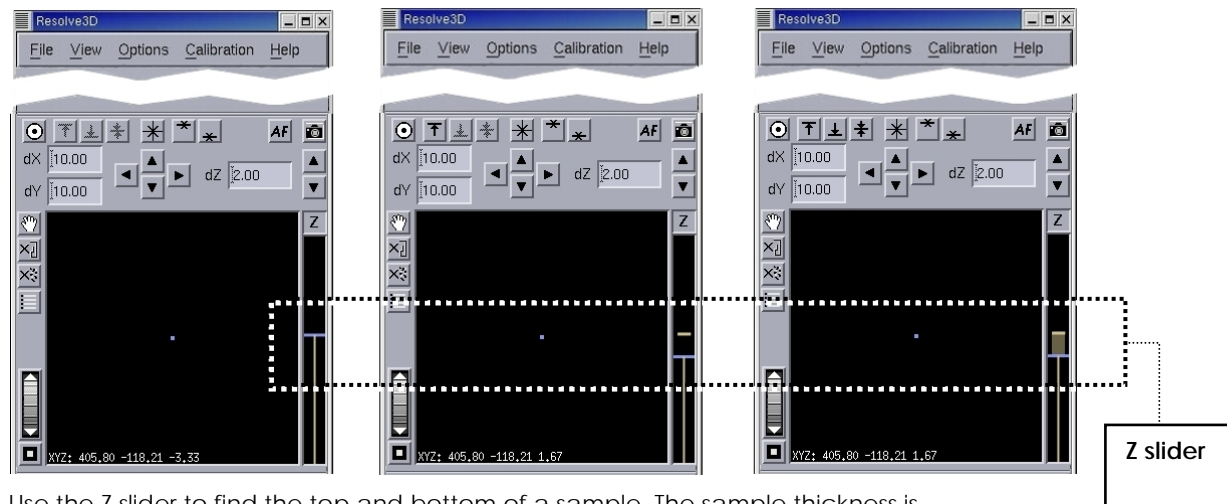
 **Note** The *softWoRx* software reports all distances in microns.

To design and run a 3D experiment:

1. Manually acquire images of your specimen and adjust acquisition parameters and settings until you are satisfied with the images. This includes focusing on the specimen and determining what channels to use.
2. In the Resolve3D window, use the Z Slider on the right side of the Resolve3D window to find and center the object of interest. Drag the slider up to find the top of your sample. (When you release the mouse button, an image is acquired

and displayed in the Data Collection window.) Drag the slider and acquire images until you are satisfied with the position. Then press the  button to mark that location. Next, use the slider to find the bottom of the sample. When you are satisfied, press the  button to mark that location.

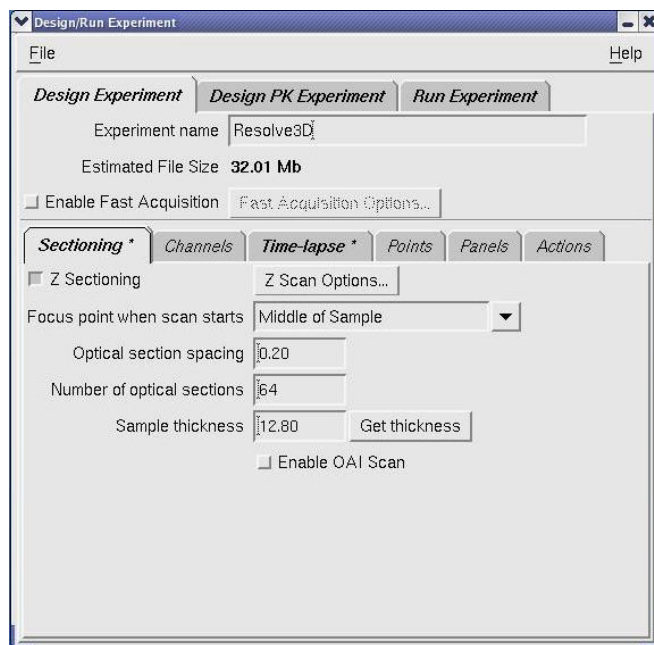
Note To reduce the risk of running the objective lens into the sample, the Z slider is limited to 5 μm at a time.






Use the Z slider to find the top and bottom of a sample. The sample thickness is indicated by the wide line on the Z slider (shown on the right image).

Note The Z stage view displays the relative position of the objective lens to the sample for an inverted microscope. Dragging the Z slider down focuses the microscope closer to the cover glass (negative direction). Dragging the Z slider up moves the focus from the cover glass toward the microscope slide (positive direction).

3. Click **Experiment** to open the Design/Run Experiment window and click the Design Experiment tab. Then click the Sectioning tab.



4. Specify the focus point of the microscope at the start of the experiment and move the stage to that focus point as follows:

Choose one of the following *focus points in the Focus point when scan starts list.	Then click the corresponding button in the Resolve3D window to move the stage to that focus point
Top of the sample	
Middle of the sample	
Bottom of the sample	
* For most applications, the Middle of the sample provides the best results.	



CAUTION: Be sure to set the focus consistently with this setting before you start the experiment. (For example, if you specify to use the Middle of Sample option, be sure to focus midway between the top and bottom of the sample.) *DeltaVision* generates the sectioning commands based on this relative starting point. If this is not done correctly, you can lose important data.

5. Specify the separation (in microns) between each optical section in the **Optical section spacing** field. If you are using a 60X objective, start by using the default value (0.2 μm). If you are using another objective, click the **Lens** button on the Resolve3D window to open the Lens Information dialog box and use the value in the **Recommended Z Step size** field (at the bottom of this dialog box). As a general rule of thumb, the Z step size is approximately 1/3 of the Z resolution for each objective.
6. Specify either the thickness or the number of sections as follows:

- To get the thickness of the specimen, as defined in Step 2, click **Get thickness**. *softWoRx* adjusts the number of sections to span the specimen thickness.
- To specify the number of sections, enter the number of sections in the **Number of optical sections** field.

7. Save and run the experiment.

Guidelines for Designing and Running 3D Experiments

Use the following guidelines to determine values for the fields on the Sectioning tab under the Design/Run Experiment window, Design Experiment tab.

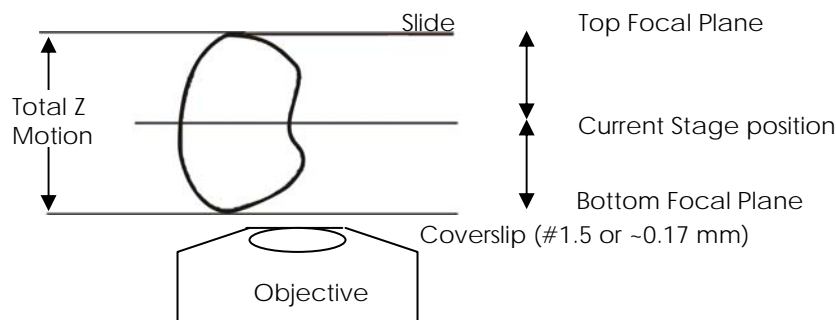
Focus point when scan starts

This list allows you to specify the focal plane of the microscope at the start of an experiment.

Choose one of the following options:

- **Middle of Sample** The focal plane is midway between the top and bottom of the sample. This is the default and is usually the "plane of best focus" for the sample. This is also the best point to set for Point Visiting.
- **Bottom of Sample** This represents a plane where the Z stage is farthest away from the objective.
- **Top of Sample** This represents a plane where the Z stage is closest to the objective.

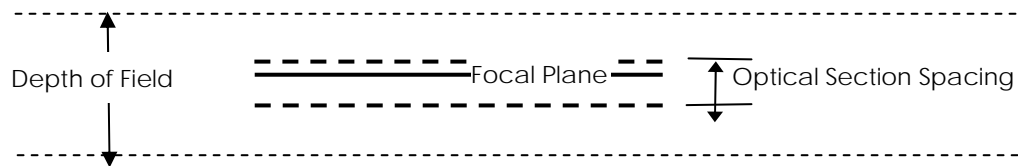
Figure 1: Z Top and Z Bottom



Optical section spacing

This parameter indirectly defines the size of the incremental Z stage movement (the distance between focal planes).

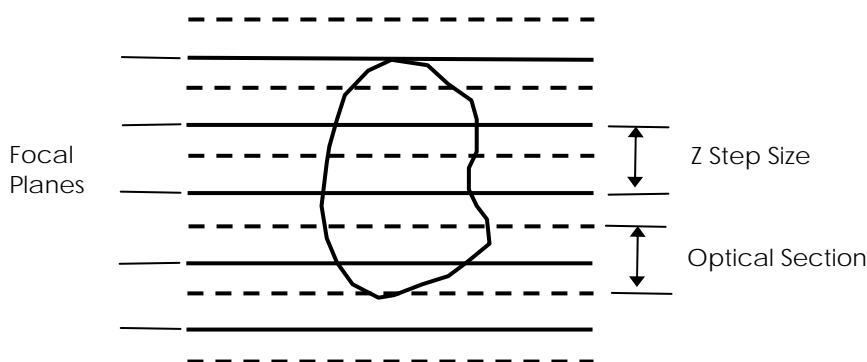
When *DeltaVision* acquires an image, the objective collects information within the depth of field above and below the focal plane.



The Optical section spacing specifies the upper and lower boundaries for which information is collected. It is centered on the focal plane and includes the information above and below that plane.

When you select an Optical section spacing, *DeltaVision* uses a Z step size that is the same distance as the Optical section spacing. (The Z step size is the vertical distance that the stage moves between focal planes.)

Figure 2: Optical Section Spacing



The standard step size for a 1.40 NA lens is 0.2 μm , although other step sizes are usually fine. Due to the inherent Z resolution of a 1.40 NA lens, there is little need to take steps finer than 0.1 μm .

When scanning a PSF, use 0.1 μm steps. For low power lenses, the step size should be appropriately larger. The Lens Info dialog for each lens contains the suggested Z Step size for each lens (derived using Nyquist sampling). This value is approximately 1/3 of the theoretical Z resolution for each objective.

Number of optical sections

This field defines the number of optical sections in the Z series of the experiment. Enter the number of sections necessary to span from the lower to the upper boundaries of the sample.

The required number of Z sections depends upon the thickness of your specimen. A typical scan consists of between 16 and 64 sections, but there is no set number.

For best results, use at least three optical sections. Using one or two optical sections, however, will also provide acceptable results.

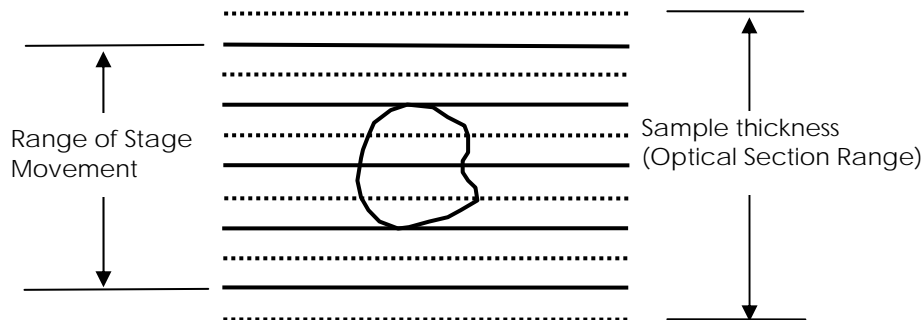
The maximum number of optical sections is limited by memory and time. Applied Precision's benchmark deconvolution consists of 64 optical sections that have XY dimensions of 512x512.

If you find it necessary to acquire many Z sections, use the following formula to figure out how much random access memory (RAM) your computer needs to efficiently perform the deconvolution. Less RAM results in slower performance.

Available RAM (bytes) > {(X size) x (Y size) x (Z size) x 4}

Sample thickness

This field displays the thickness of the optical section range. It is approximately one section thicker than the actual stage movement, as shown below.







For optimal deconvolution, the Z sections should extend beyond the volume of interest by 1-3 sections.

Sample thickness = Optical section spacing x Number of optical sections




A number of methods have been used to determine the necessary Z scan settings. The best method for your work depends largely on preference and experience, and there is not really a best way to approach the problem. Listed below are some of the methods that have been used:

- **Exact Method:** Use the exact method when you want to make a perfect Z scan. Use the position buttons in the middle of the Resolve3D window to find and center the object of interest as follows:

Use the  button to move the Z focus to the top of the sample and mark the top location with the  button. Then use the  button to move to the bottom of the object and mark the bottom location with the  button. On the Sectioning tab, use the **Get thickness** button to calculate the thickness of the sample. (The thickness is entered into the **Sample Thickness** field). Note that the experiment created by Design Experiment uses relative motion for Z and

not absolute coordinates. All motion in Z is relative to the starting point and does not refer to an absolute Z position.



Note The top and bottom can be viewed by pressing the  button or the  button respectively. The middle section can be viewed by pressing the  button.

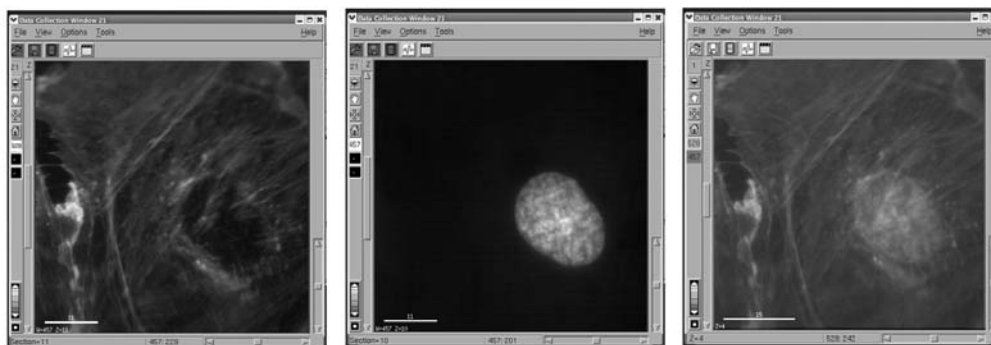
- **Overscan Method:** As the name implies, the overscan method involves using a Z range that is much greater than the range of interest. For example, design an experiment that scans double the expected Z range. Previous experience is obviously helpful when using this method.

When using this method, it is recommended that you figure out which Z sections contain the desired information before performing the deconvolution. Cut out unnecessary Z sections during the deconvolution step of the image processing by specifying the section numbers of the Z start and Z end. This will help to ensure that the sample is imaged.

- **Previous Experience:** This method is essentially the same as the overscan method, except that one strives for a more exact scan. For example, if you are scanning an erythrocyte with $7\mu\text{m}$ diameter, you could focus on the center of the cell, and then scan $4\mu\text{m}$ above and $4\mu\text{m}$ below the cell. Of course, if you underscan the object, then you might miss important details, so it helps to be conservative.

Selecting Filters

From the Channels tab of the Design/Run Experiment dialog box, you can define which filter sets to use in the experiment for imaging. *DeltaVision* acquires images from each filter set (channel) and creates a single *DeltaVision* file that contains all of the channels that were collected. There can be up to five channels per Experiment.



FITC (left) and DAPI (center) filtered images are combined in the final *DeltaVision* file (right)

Choosing which filters to use

Your choice of filters will be determined by the types of fluorescent probes with which you have labeled your sample and the filters available on your system. The excitation and emission spectrum of your filters should match the spectra for the absorption and emission of the fluorescent probes you want to use.

The following tables show which *DeltaVision* filters match each particular probe. The interchangeable Standard and Live Cell filter modules are available for both *DeltaVision Core* and *personalDV*. The Standard filter module filter sets are typically used for fixed specimens while the Live Cell filter module sets are commonly used for live specimens.

Figure 3: Standard *DeltaVision* Module Filter Sets

Probes	Filter Name	Excitation	Emission
DAPI, Hoechst, Coumarin	DAPI	UV, 350nm	Blue, 455nm
Fluorescein, GFP, CY2, Al488	FITC	Blue, 490nm	Green, 525nm
Rhodamine, Texas Red, Cy3,	TRITC	Green, 555nm	Orange, 605nm
CY-5®	CY-5	Red, 645nm	Infrared, 705nm

Figure 4: Live-cell Module Filter Sets

Probes	Filter Name	Excitation	Emission
Cyan GFP	CFP	430nm	470nm
Yellow GFP	YFP	500nm	535nm
mCherry	mCherry	572nm	632nm
EGFP, sgGFP	EGFP	470nm	525nm

You can select other combinations of excitation and emission filters. You can also add your own custom filter sets. To determine the optimal filters for your application, compare the excitation and emission spectrum of your filters to the spectra for the absorption and emission of the fluorescent probes that you want to use.

The spectra for the standard *DeltaVision* filter set and the optional Live Cell set are provided in Appendix D.

Notes

#1 The lists of probes in Figure 3 and Figure 4 do not include all of the probes that can be used with these filter sets.

#2 Additional filter sets may be purchased from Chroma Technologies or Semrock.

#3 The CY-5 filter set does not include an eyepiece filter due to the low sensitivity of

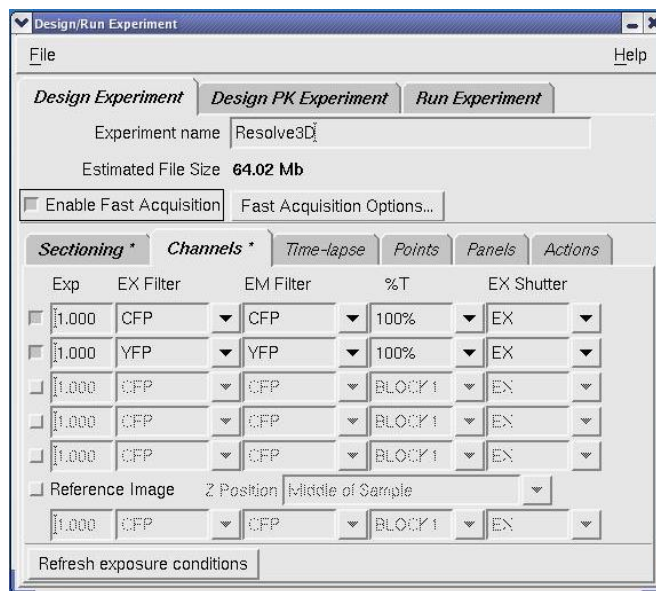
the human eye at this wavelength.

#4 The standard configuration for the six position filter wheels includes: DAPI, FITC, TRITC, CY-5, a polarizer, and a block position.

For Instructions on to change filter wheel modules and calibrate filter wheels, see *Changing Filter Wheel Modules* on Page 114 and *Calibrating the Filter Wheels* on Page 121.

To specify which filters to use:

1. On the Design/Run Experiment window, click the Design Experiment tab. Then click the Channels tab.
2. Select a check box on the far left next to an **Exp** field. The **EX Filter** field controls which excitation filter will be placed into the excitation pathway between the %T filter and the fiber optic module. The %T Filter attenuates the intensity of the excitation light. The **EM Filter** field controls which filter will be placed in the emission pathway between the microscope and the camera. The **EX Shutter** field specifies which light source to use for that channel.
3. On the **EX Filter** list for the same line as the check box you selected, select the excitation filter. The EM Filter (emission filter), %T Filter (neutral density filter), and eyepiece filters for that filter set are automatically specified. (*DeltaVision* specifies the most recent filters that were selected for that filter set in the Resolve3D window. If no filters have been selected, *DeltaVision* specifies default filters for that filter set.)



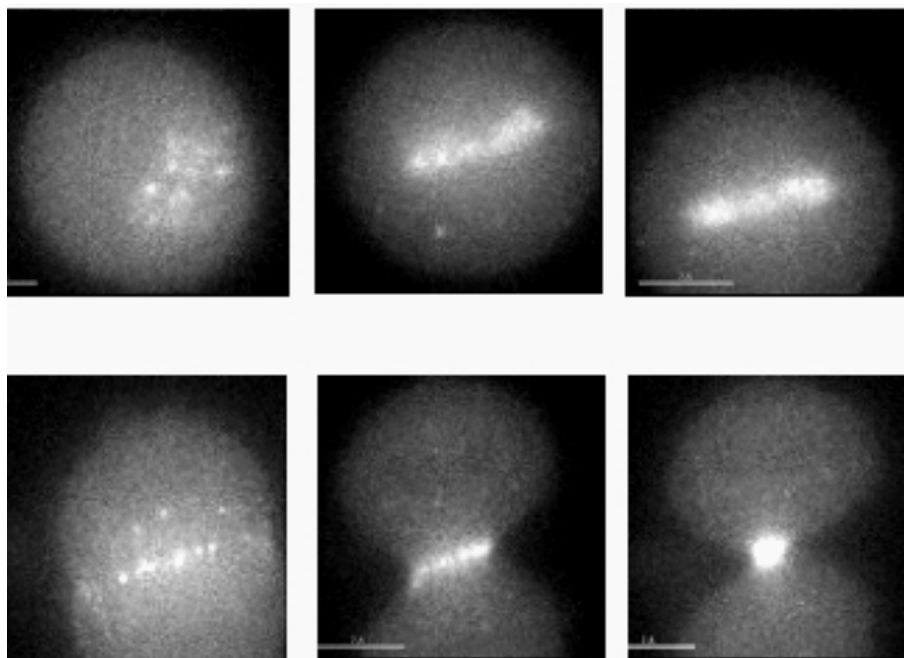
4. To change the exposure time, enter a value in the **Exp** field. Values in the Exp field are in seconds. For example, 0.100 is 1/10th of one second. If you do not

enter a value in this field, the most recent exposure value is automatically set for each filter.

5. To select a different emission filter or to attenuate the intensity, choose a filter from the **EM Filter** or the **%T** lists.
6. You can choose which light source to use for each channel by selecting a shutter from the **EX Shutter** field. **EX** is the standard fluorescence light source, **TRANS** is the transmitted light source, **EX2** is an optional second fluorescence light source, and **LASER** is an optional laser source using the QLM module.
7. If you are repeating an experiment and you have entered new exposure times or %T in the Resolve3D window, click **Refresh exposure conditions**.
8. Repeat Steps 2-6 for each set of filters that you want to use.
9. Save and run the experiment.

Setting up Time-Lapse Experiments

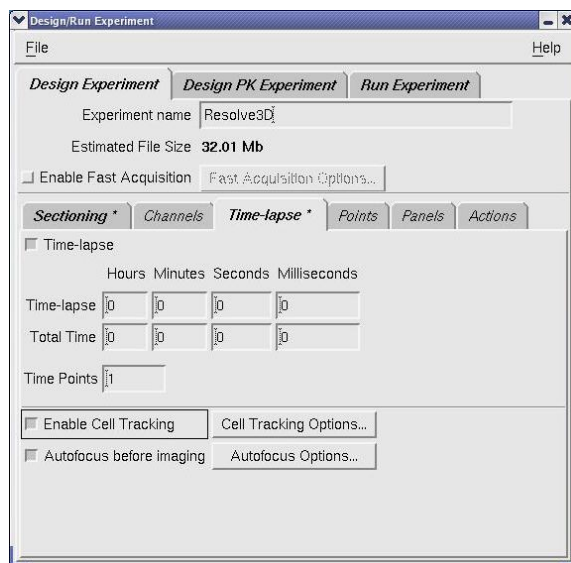
Time Lapse images are very useful for analyzing live specimens.



Time-lapse images showing cell mitosis

To design a time-lapse experiment:

1. Select the Time-lapse tab under the Design Experiment tab on the Design/Run Experiment window.



2. Enter the desired time interval in the **Time-lapse** field.



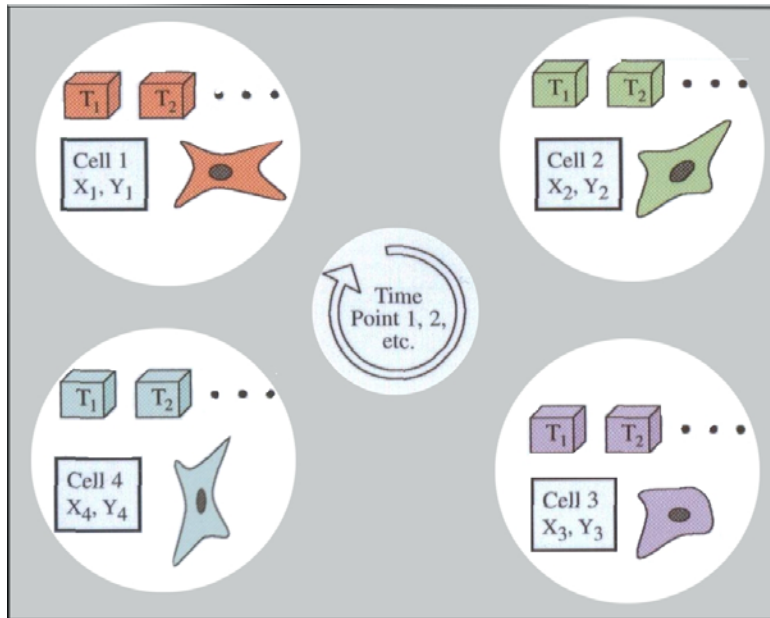
Note The minimum time interval is limited by acquisition time. If you specify a time that is less than the acquisition time, the experiment proceeds at the quickest possible rate.

3. Set up the remaining parameters for your experiment in one of the following ways:
 - Enter the desired number of time points in the **Time Points** field. The **Total Time** field displays the total time that will elapse during the experiment.
 - Enter the total time in the **Total Time** field. The **Time Points** field displays the number of time points for the experiment.

Point Visiting

You can use point visiting to monitor areas of the slide that are in different fields of view. Instead of recording one cell or field in a single experiment, multiple sites can be imaged in a single experiment, increasing data collection efficiency.

In practice, the number of sites is limited only by the minimum acceptable time interval between each time point at a single site. This makes time-lapse imaging much more efficient, and allows you to collect enough data to generate statistically significant results. In addition, variability between cells within an experiment can be assayed, eliminating uncertainty as to the behavior of cells in a single experiment.



Point Visiting monitors points that are in different fields of view

To set up an experiment that visits points, you must:

- Mark the points to visit with the Keypad or with Resolve3D and save a point list.
- Edit the point list if necessary.
- Load the point list and specify which points to visit.

Notes

#1 If you are placing your slide directly on the microscope stage (without the Repeatable Slide Holder), you can store the X, Y, and Z coordinates of the stage positions you mark; however, if the slide is removed from the stage or the Z focus knob is adjusted, these coordinates no longer apply.

#2 If you are using Applied Precision's Repeatable Slide Holder, you can remove the slide and then place it in the same position when you want to revisit the points. To do this, you must record the position (A-G) of the slide before you remove it. Then put the slide in the same position when you return it to the slide holder and update the Z coordinates.

Marking Points

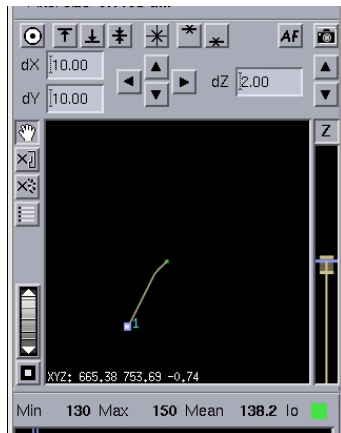
There are two ways to mark points within the sample: using the keypad or using Resolve3D. An efficient way to mark points is to use both methods together. First, mark the points using the keypad, which is quick and approximate, and save the point list. Then, in Resolve3D, specify a more exact location for each point.

The points marked using the keypad are automatically communicated to Resolve3D and displayed in the Stage view. If you open the Point List dialog, you will see the list of points.

To mark points using the keypad and Resolve3D:

1. Use the arrows on the keypad or the joystick to move to the desired point.
2. Press the POINT MARK key on the keypad.

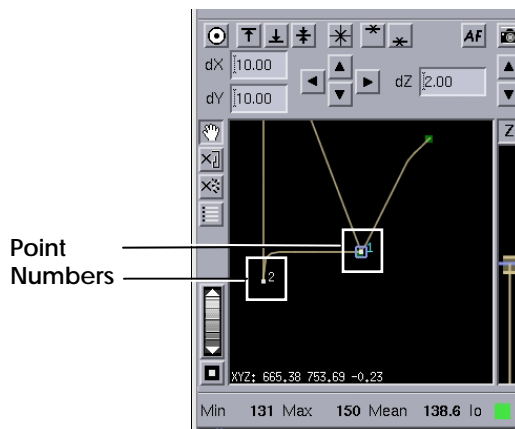
The number of the marked point is displayed in the Stage view.



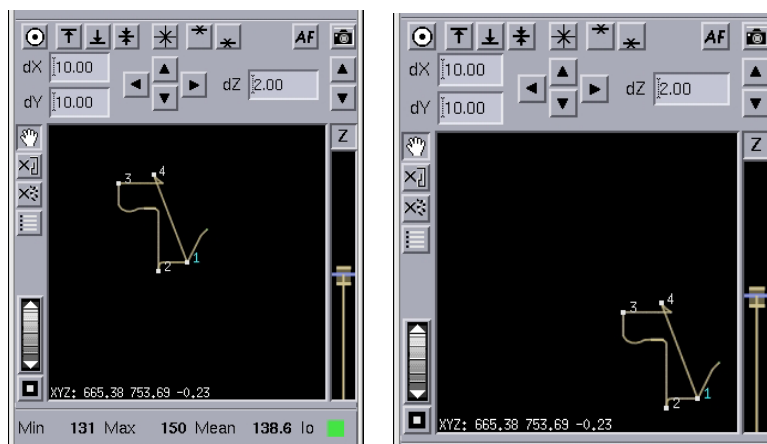
Marked point in the Stage View

3. Repeat steps 1 and 2 to mark the desired points.

As you mark points, the stage trails and points (along with their point numbers) are displayed in the Resolve3D Stage view.

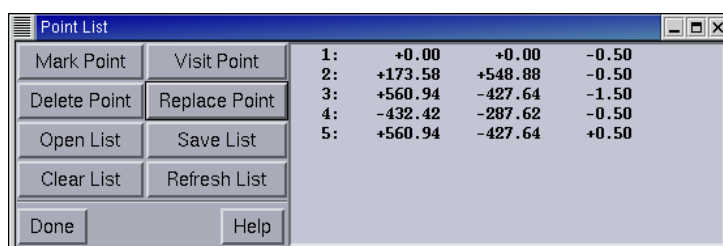


4. To view all of the points, use the Zoom Wheel to zoom out on the stage view. To pan the stage view, use the  button.



The stage view after zooming in (left) and panning (right)

5. In the Resolve3D window click  to open the Point List dialog box.



6. Click on a point to select it and then click **Visit Point** to move the stage to that point.
7. Use the XY Stage Controls on the Stage View to move the stage to the exact XY position.
8. Use the Fine Z Focus knob to focus while looking at the sample through the eyepieces. Then acquire an image to execute an LMC move and verify that the image is in the best focal plane. Repeat this step if necessary until the image is in focus.



XY Stage Controls

! **Important** Do not use the microscope Focus knob after you complete Step 8. Instead, use the Resolve3D Z controls (the Z buttons on the Resolve3D window or the Z slider) or the **AF** (autofocus) button.

9. On the Point List dialog box, click **Replace Point** to replace the old coordinates with the new exact coordinates for the point.
10. Repeat Steps 6-9 to replace all of the points in the list with the exact X, Y, and Z coordinates.

Editing a Point List

To delete a point using Resolve3D:

1. In the Point List dialog box, select the point that you want to delete.

2. Click **Delete Point**.

To save the point list:

1. In the Point List dialog box, click **Save List**.
2. In the Prompt dialog, enter the desired name for the list, using eight characters or less. Since there is really no method of displaying a directory of these names, write the name of the list down to keep a record of it.
3. Press Enter or click **OK**.

To visit a point using Resolve3D:


Use one of the following methods to visit a point:

- In the Stage view, double click on the point.
- In the Point List dialog box, click the point that you want to visit and click **Visit Point**.


Loading a Point List and Specifying Points to Visit


If you are using a previously saved point list, you will need to load the point list in Resolve3D before you specify which points to visit.

If you are using a point list that is already open in the Point List dialog box, you can specify which points to visit.

 **Tip** You can mark and then visit a point list without saving it when you are setting up an experiment.

To load a point list into Resolve3D:

1. On the Resolve3D dialog box, click  or choose **View | Points List** to open the Point List dialog box.
2. Click **Open List**.
3. In the Prompt dialog box, enter the name of the desired list and click **OK**.

 **Note** You may need to load a list when the list of points has been cleared from the Point List dialog and you want to reload the saved list.

To specify which points to visit:

1. Make sure that the point list is loaded in the Point List dialog box as shown above.

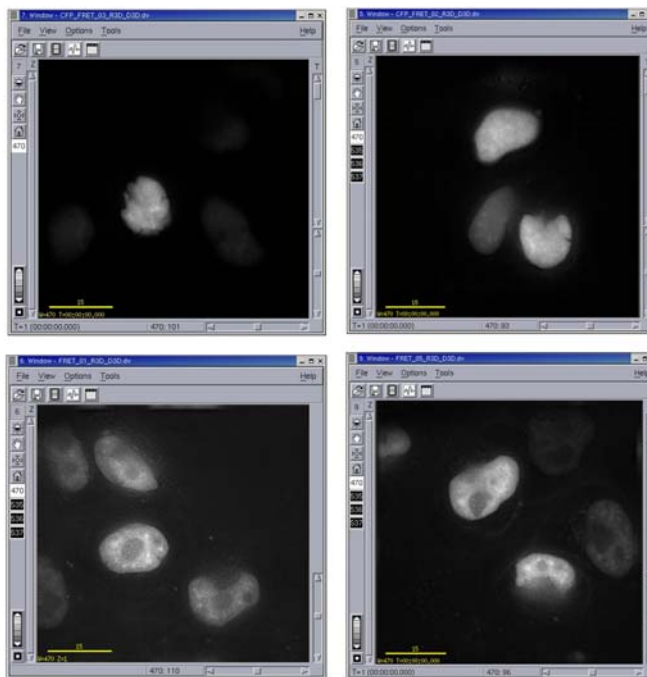
2. Open the Design/Run Experiment window and click the Design Experiment tab. Then click the Point Visiting tab and check the **Visit Point List** option.

The Point Visiting dialog box is linked to the point list that is open.

3. Enter the points that you would like to visit in the **Visit Point List** field. (For example, entering 1-3 specifies to visit points 1, 2, and 3. Entering 1-3, 5-7, 9 specifies to visit points 1-9 but not 4 or 8.)

Monitoring Point Visiting Experiments

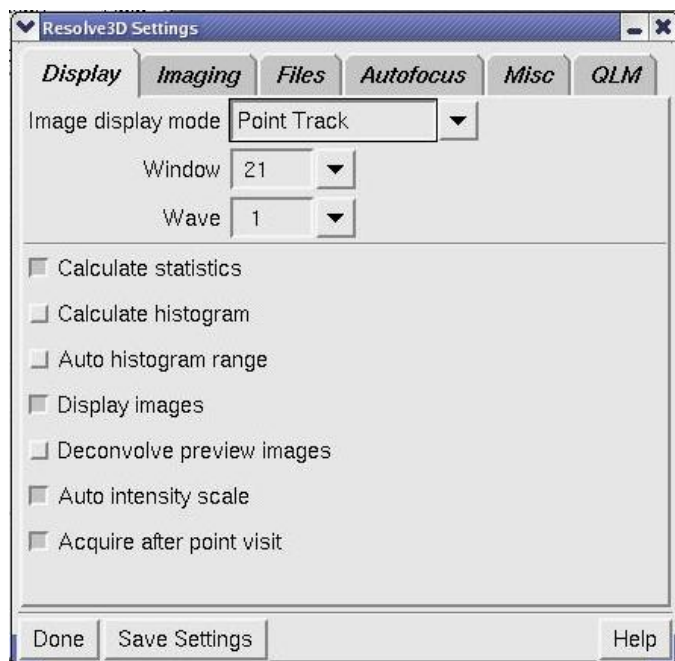
You can set up *DeltaVision* to display a separate Data Collection window for each point in your point visiting experiment.



With the Point Track Display Option enabled, each point in a point visiting experiment is displayed in a separate window

To set the Point Track display option:

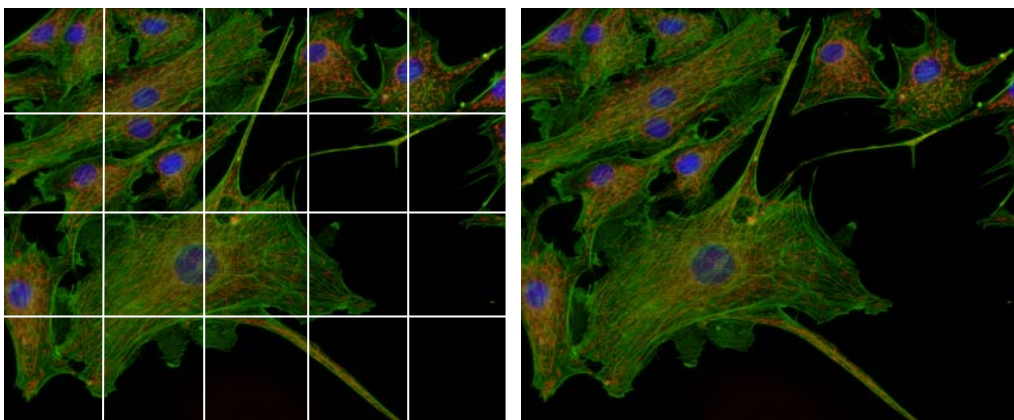
1. On the Resolve3D window, click the **Settings** button to open the Resolve3D Settings dialog box.
2. Click the Display tab.



3. In the Image Display mode list, select **Point Track**.

Collecting Panel Images Over Large Areas

Panel collection macros are useful when you want to scan a large area with a relatively high magnification lens. You can use the panels as a means of reviewing a large area of a slide, or as data that you want to stitch together to form a single, large image.



A set of panel images (left) can be stitched together to create a single image (right)
Image files that are collected with Panel Collection Setup enabled have a .pnl extension. These images must be stitched to create *DeltaVision* image files. (See *Stitching* in the *softWoRx Imaging Workstation User's manual*.)



CAUTION: Do not try to reuse panel collection experiment macros. These macros are sensitive to microscope settings such as image size and magnification because the number of panels required depends upon many factors.

Determining Border Rolloff Voxels

Before you collect panel images, you must first determine the number of voxels in the Border Rolloff that is used in Deconvolution.

 **Note** Conceptually, a voxel is a three-dimensional pixel. So, while a pixel generally refers to XY data, a voxel includes information from the Z (or depth) plane.

To determine Border Rolloff Voxels:

1. Determine the size of the panels that you plan to collect. (Smaller panels work best because they have the flattest intensity distribution.)
2. From the main *softWoRx* window, choose **Process** | **Deconvolve** to open the Deconvolve window. In the **Input** field, enter the file name of an image that is similar to the panel size. (You can acquire and save a blank image of that size for this purpose.)
3. Click the **More Options** button to open the More Deconvolution Options window and record the value that is displayed in the **Border Rolloff (voxels)** field.

Collecting Panel Images

To collect 3D panel images:

1. From the Resolve3D window menu, choose **View** | **Point List**. In the Point List dialog box, choose **Mark Point** and mark points in two opposite corners to define the area of your final composite image.
2. In the Resolve3D window, click **Experiment** to open the Design/Run Experiment window.
3. Click the Design Experiment tab. Then set up the Sectioning and Channels information in the same way that you would for standard data collection.
4. In the Point List dialog box, select one of the points that you marked in Step 1 and click **Visit Point**.
5. Click the Panels tab under the Design Experiment tab. Then select the **Collect Panels** option.

6. Click **Get Start** under the Panels tab. Then visit the other point that you marked in Step 1 and click **Get End**. (Alternatively, you can enter the coordinates that are displayed in the Point List dialog box.)
7. In the **Overlap (pixels)** field, enter at least twice the number of border rolloff voxels as you recorded when you determined Border Rolloff voxels (see Page 51). If you generally adjust the pixel size to <1 or use rotation when stitching, use a larger overlap. (The spacing information is automatically updated.)
8. Save and run the experiment.

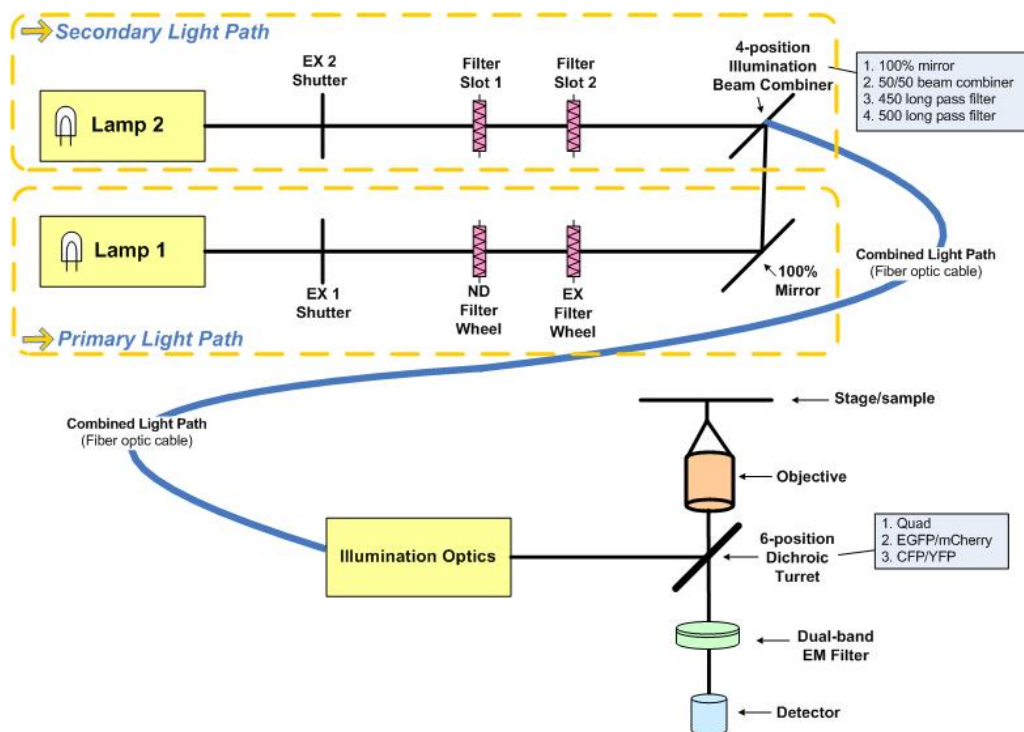
For instructions that show how to use *softWoRx* to stitch panels together, see the *softWoRx Imaging Workstation User's Manual*.



Note You cannot easily use Panel Collection with time-lapse or point-visiting experiments. It is possible only by editing the header.

Using the Multiplexed Wavelength Option

The optional Multiplexed Wavelength module for the *DeltaVision* system allows you to perform nearly simultaneous two-channel imaging without the drawbacks associated with true simultaneous two-channel imaging. This option uses two shuttered illumination sources and a dual-band emission filter to eliminate filter wheel movement between channels, and therefore greatly reduces the time required for the *DeltaVision* system to acquire a set of two-channel images. The combined light path ensures no registration artifacts are introduced and independent excitation of probes helps to ensure minimal crosstalk.



Conceptual view of Multiplexed Wavelength functionality

Before you use the Multiplexed Wavelength option, you must first have it installed and configured correctly. Your Applied Precision representative will assist you in setting up this option and help you ensure that all hardware and software to support Multiplexed Wavelength functionality is installed properly.

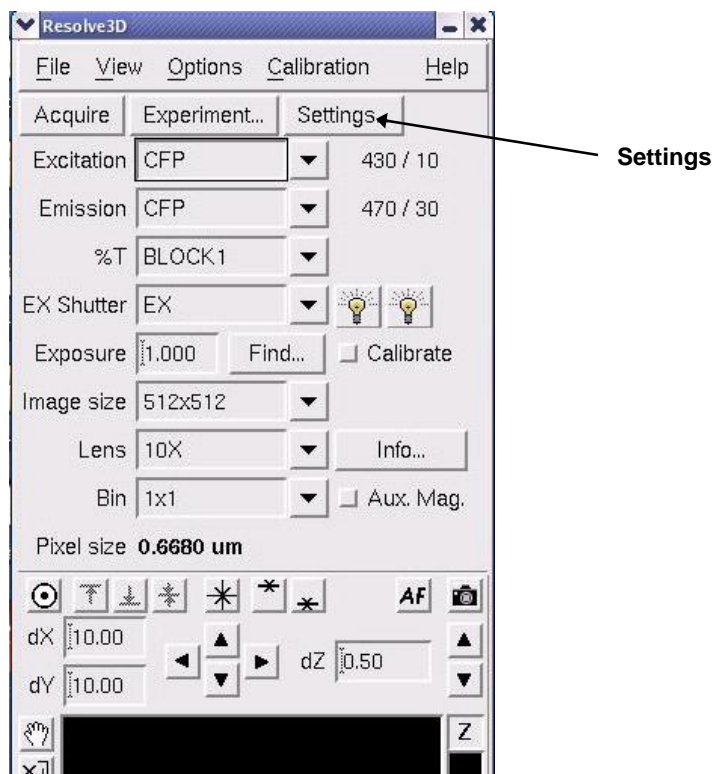
After the option has been installed and configured, the menus, tools, and other infrastructure necessary to use the feature will be available on your workstation.

Setting Up the Multiplexed Wavelength Option

Before you begin designing your Multiplexed Wavelength experiment, you'll need to perform the steps described in the following procedures to activate a Multiplexed Wavelength filter set and prepare the DeltaVision system for Multiplexed Wavelength operation.

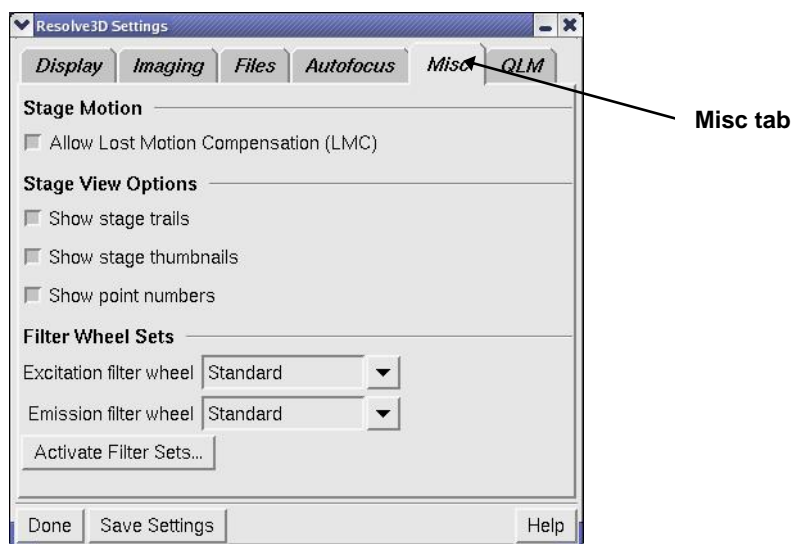
To activate the Multiplexed Wavelength filter set:

1. To change the active filter set to a filter set that is Multiplex capable, select **Settings** in the Resolve3D main menu.

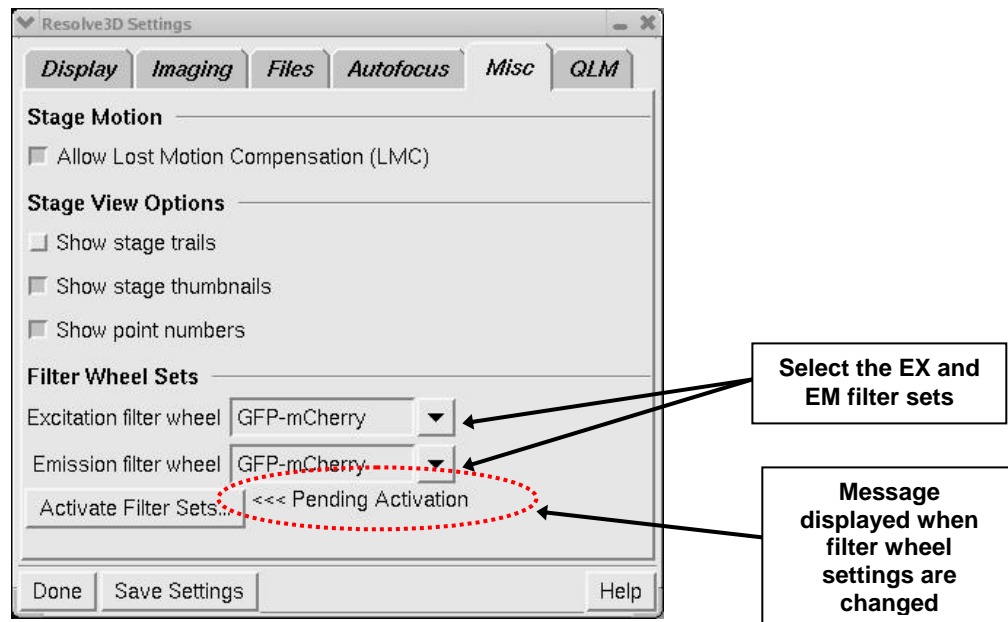


The Resolve3D Settings window is displayed.

2. In the Resolve3D Settings window, click on the Misc tab.

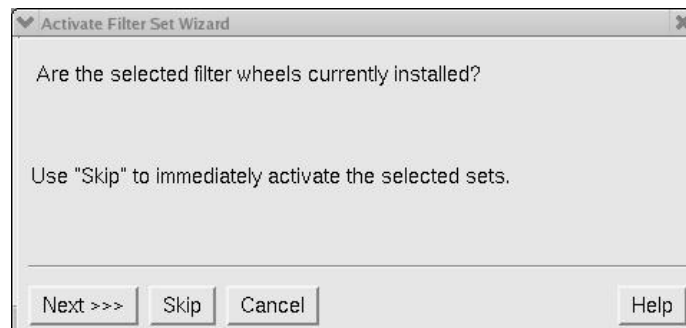


3. In the Resolve3D Settings window, select the EX and EM filter sets you want to use. When these fields are changed, the <<<Pending Activation message is displayed in the window (as shown).



4. Click **Activate Filter Sets**. The following confirmation window is displayed.

Note If you select filter sets for the Excitation filter wheel and Emission filter wheel fields and then click **Done** in this window, your selections are retained until you either activate the filter sets or exit Resolve3D.



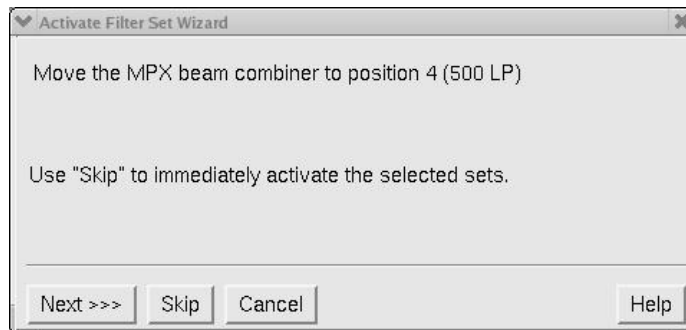
Note For the filter sets to be activated, the selected multiplexed filter set filters must exist in the currently installed excitation and emission filter wheels. To determine the proper filter sets, refer to the "Changing Filter Wheel Modules" section in Chapter 8.

5. If the selected filter wheels are installed on your *DeltaVision*, click **Next** to continue.

Note If you click **Skip** from this window, the selected filter wheels are activated immediately and the remainder of the activation wizard is skipped.

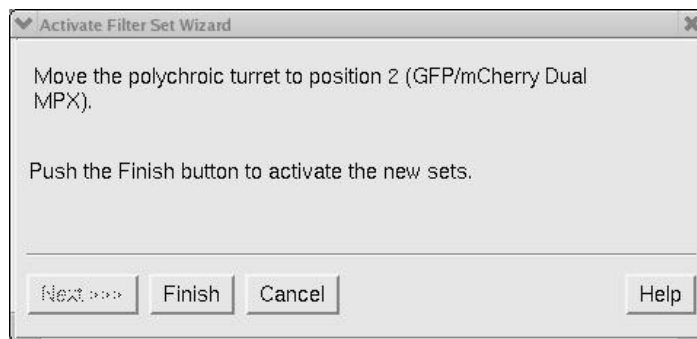


6. Insert the selected secondary filter insert (mCherry is used in this example) into Filter Slot 1 of the secondary light path and click **Next** to continue.



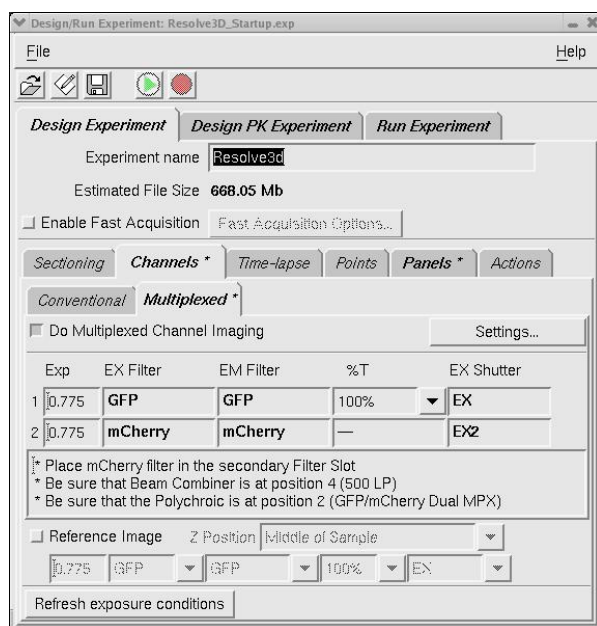
The system gathers the information for this window (in this case, "position 4 (500 LP)") from the `MXWSetup.ini` file, not from the Instrument Controller.

7. Move the beam combiner to the appropriate position and click **Next** to continue.



Again, *softWoRx* gets the information for this window (in this case, "position 2 (GFP/mCherry)") from the `MXWSetup.ini` file, not from the Instrument Controller.

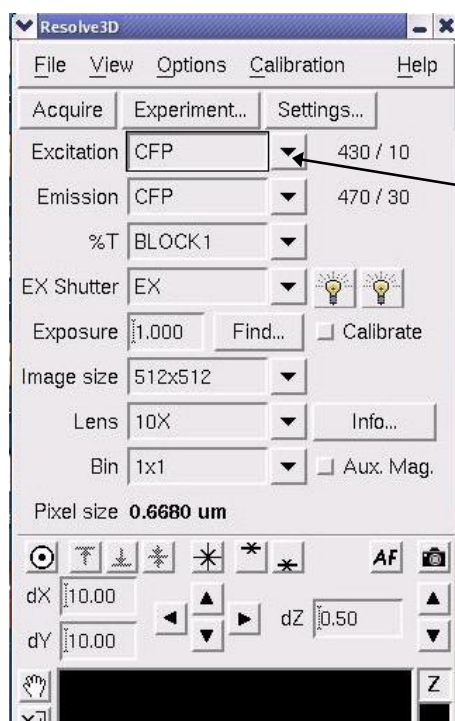
8. Click **Finish** to complete the Multiplexed Wavelength filter activation process. After the selected filter set is activated, the Design/Run Experiment window will look similar to the following.



At this point, you have completed activating the Multiplex Wavelength filter set. You should now continue with the steps in the next procedure for viewing a sample with the Multiplexed Wavelength operation.

To view a sample using the Multiplexed Wavelength option:

1. Rotate the eyepiece filter wheel to the POL or BLANK position.
2. From the Resolve3D main menu, select the Excitation filter currently in the primary light path (CFP or GFP).



Select the EX filter currently in the primary light path.

3. Use the EX button on the keypad to open the primary EX shutter and view the primary light path.
4. Use the EX2 (formerly CAMERA SHUTTER) button on the keypad to open the EX2 shutter and view the secondary light path.



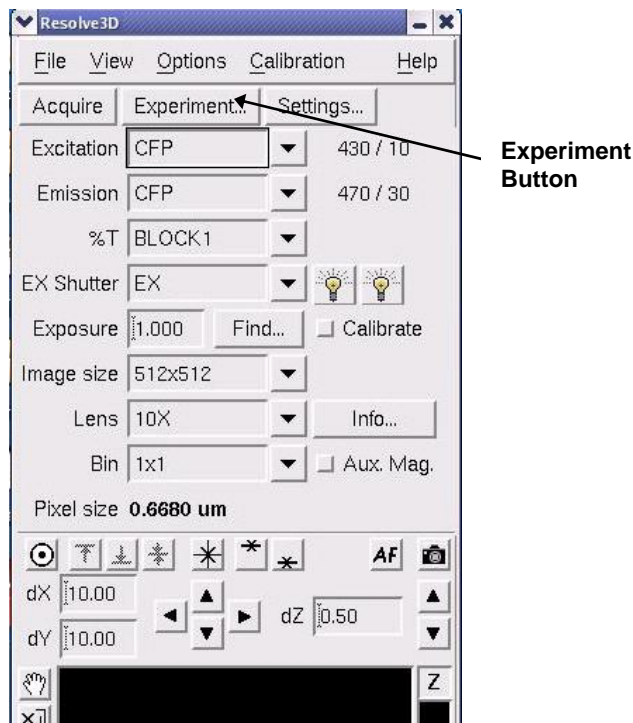
Note With the Multiplexed Wavelength option, you can view both selected wavelengths simultaneously by opening both EX shutters at the same time.

Designing a Multiplexed Wavelength Experiment

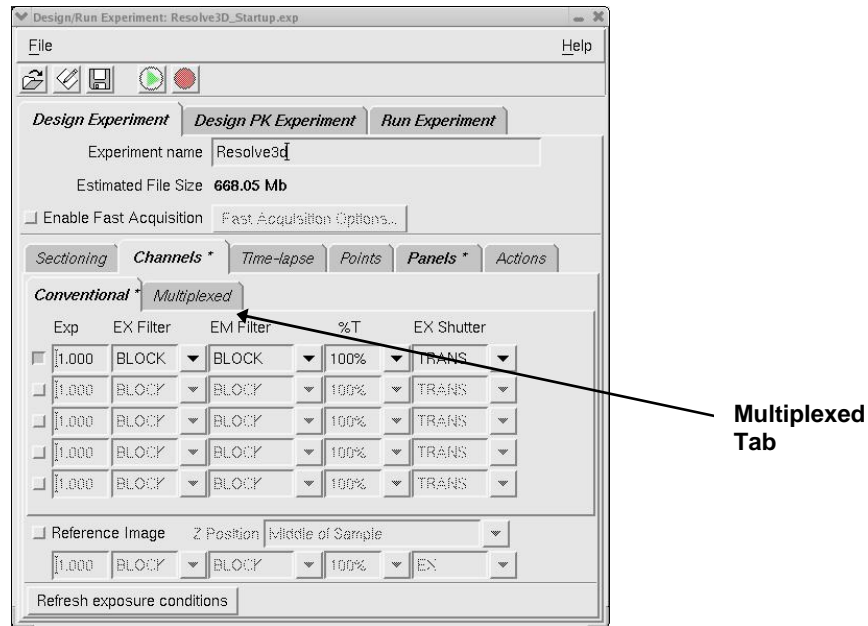
Use the following procedure to begin the design process for Multiplexed Wavelength experiments.

To design a Multiplexed Wavelength experiment:

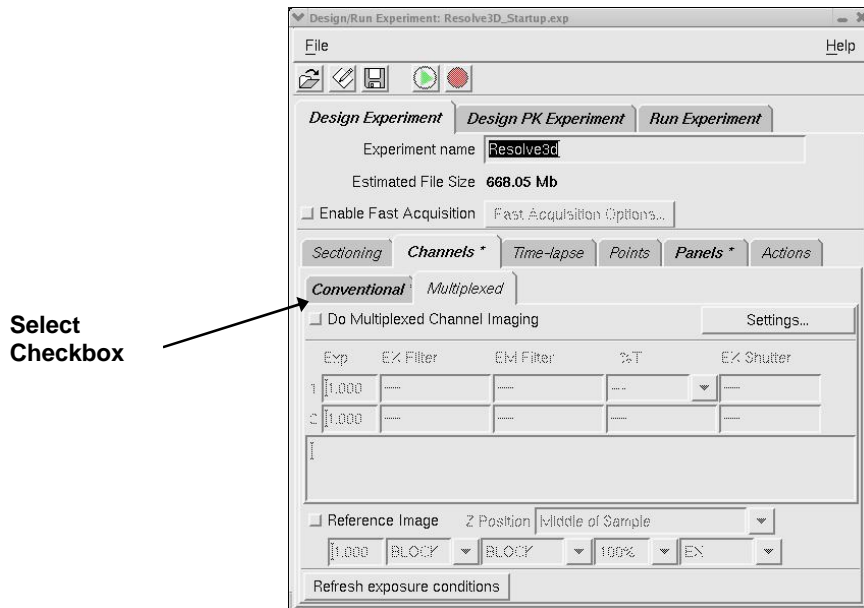
1. From the Resolve3D main menu, click the **Experiment** button to open the Design/Run Experiment window.



2. If the Multiplexed Wavelength option is enabled on your *DeltaVision* system, you'll see the Multiplexed tab in the Design/Run Experiment window. Click on the Multiplexed tab to view the options for Multiplexed Wavelength experiments.

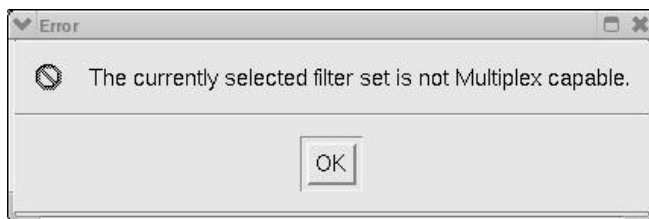


The Multiplexed tab of the Design/Run Experiment window is displayed as shown.



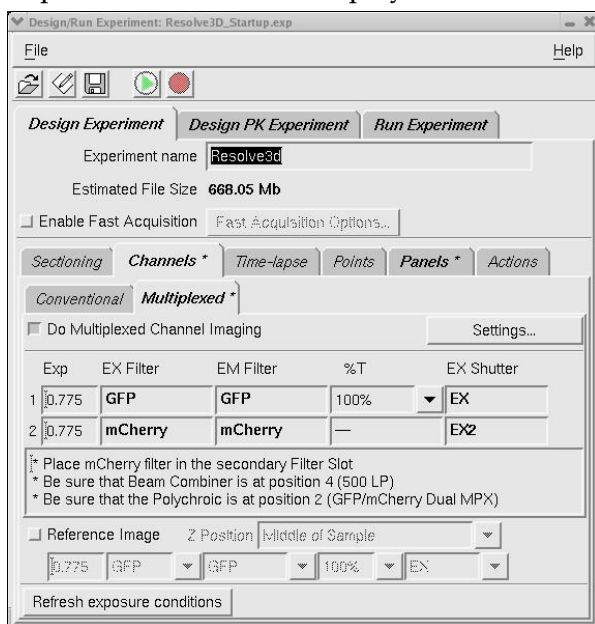
- From the Multiplexed tab, select the **Do Multiplexed Channel Imaging** checkbox.

If the currently active filter set is *not* Multiplex capable, the following window is displayed.



You will need to change the active filter set to continue. Press **OK** to return to the Design/Run Experiment window. To change the active filter set for Multiplexed Wavelength experiments, see the procedure for activating a multiplexed wavelength filter set in “Setting Up the Multiplexed Wavelength Option.” (Also see “Setting Up Filter Wheels” in Chapter 8 for additional information on changing the active filter set.)

If the currently active filter set *is* Multiplex capable, the Design/Run Experiment window is displayed and will look similar to the following.



At this point, you have completed the initial portion of the Multiplexed Wavelength experiment design setup. You should now continue with the standard steps for the remainder of the experiment design, such as Sectioning, Timelapse, and so on.

Note As soon as you activate the **Do Multiplexed Channel Imaging** checkbox, all conventional imaging settings are disabled. This is also true of all multiplexed settings when you reactivate conventional imaging.

5

Acquiring Data From Live Specimens

This chapter shows how to use the following features to image live cells with *DeltaVision*.

- Set up experiment macros to automatically focus before acquiring each image. This is useful for cells that move during the experiment.
- Use Cell Tracking to follow cells as they move laterally and move the stage to keep them in the field of view.
- Use Optical Axis Integration (also referred to as Z Sweep Acquisition) to acquire a 2D projection. This method collects and integrates one continuous image through an extended Z movement. Z Sweep Acquisition is much faster than the traditional technique of collecting an individual image at each focal plane. It also reduces the risk of specimen damage and has less total camera read noise.
- Acquire reference images that can be used for Differential Interference Contrast (DIC) and other types of analysis.

Using Autofocus in Experiment Macros

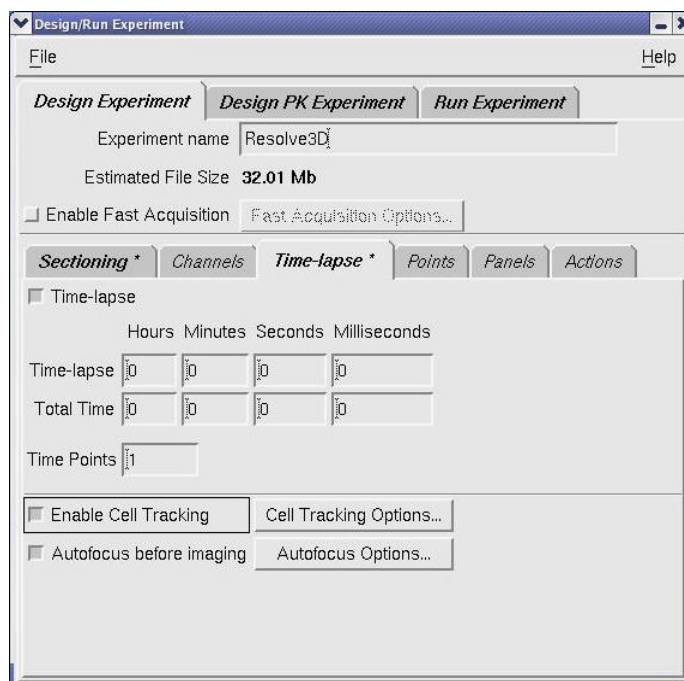
You can set up your experiment macro to automatically focus before each image is acquired. This is especially useful for long time-lapse experiments that are susceptible to changes in environmental conditions. Autofocus allows you to acquire focused images without closely monitoring the experiment. The DeltaVision Autofocus algorithm is a software-based method that uses both image contrast and peak image intensity to determine when the specimen is in focus.

To optimize the focus position for DeltaVision, you must find the Z position where the contrast is greatest. However, this position may not always be at the optimal, in-focus plane of the sample. Autofocus allows you to adjust several parameters within the standard algorithm so that the final Z position is most favorable for the specific sample. Limiting the step size and maximum Z range of travel can limit the effects of objects that are not of interest. In cases where the contrast outside of the actual middle of the sample is highest, you can create an offset so that the middle of the sample is located during the Autofocus process.

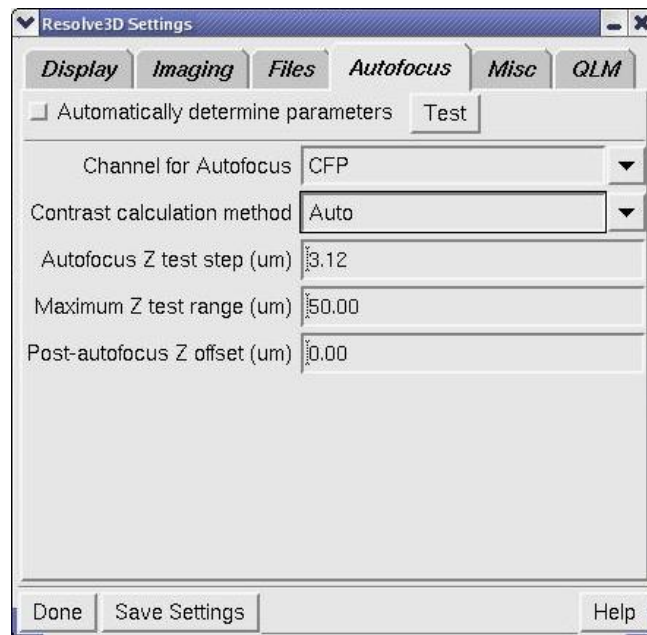
The Autofocus option works the same way as the **AF** button on the Resolve3D window. Use the following instructions to include this option in the experiment macro.

To set up an experiment that automatically focuses before acquiring points:

1. At the top of the Resolve3D window, click the **Experiment** button to open the Design/Run Experiment window.



2. Click the Design Experiment tab and then click the Time-lapse tab.
3. Select the **Autofocus before imaging** option. When this option is selected, the software automatically determines the parameters for the Autofocus process.
4. To change the various Autofocus parameters, click the **Autofocus Options** button. The Resolve3D Settings dialog box is displayed with the Autofocus tab selected.



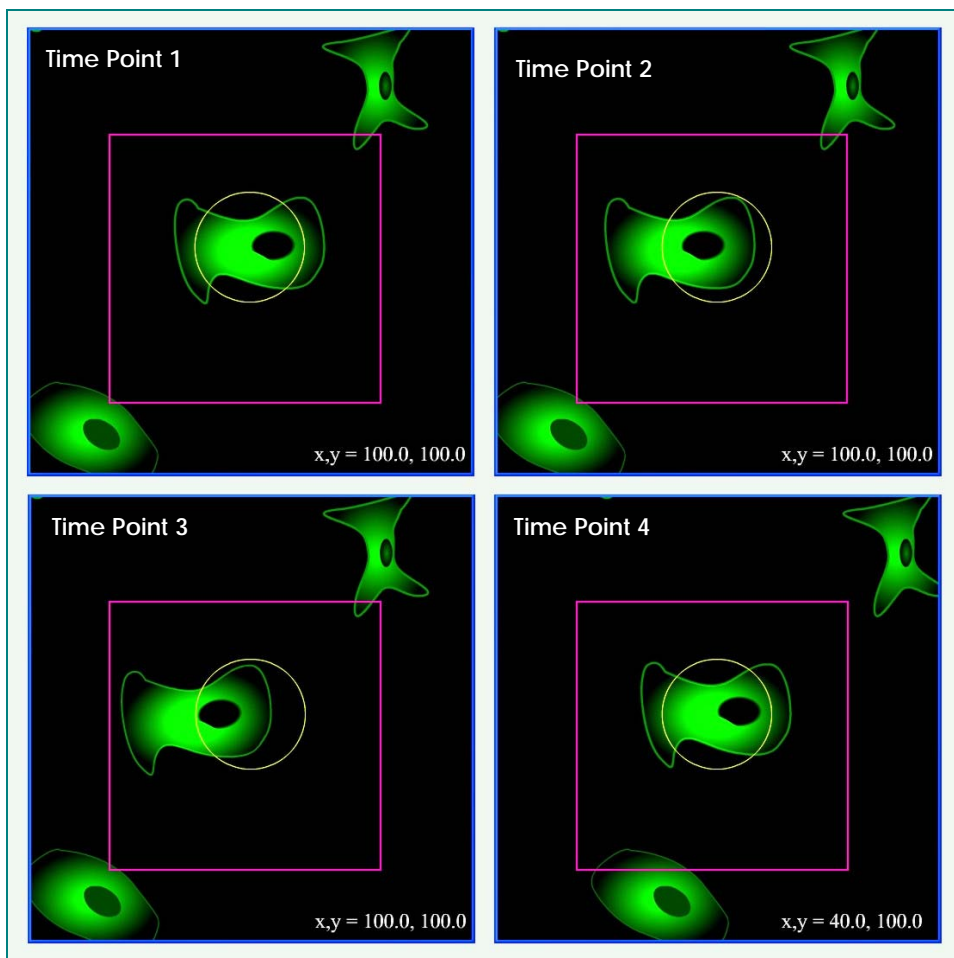
5. Deselect the **Automatically determine parameters** option. The remaining parameters on the dialog box become active.

The Autofocus parameters in this dialog box are described in Settings Dialog Box Autofocus Options on Page 210.

Tracking Cells

Cell Tracking moves the stage laterally to follow cells as they move during a time-lapse experiment. With the Enable Cell Tracking option selected, *DeltaVision* automatically keeps cells in the field of view as they move during a time-lapse experiment.

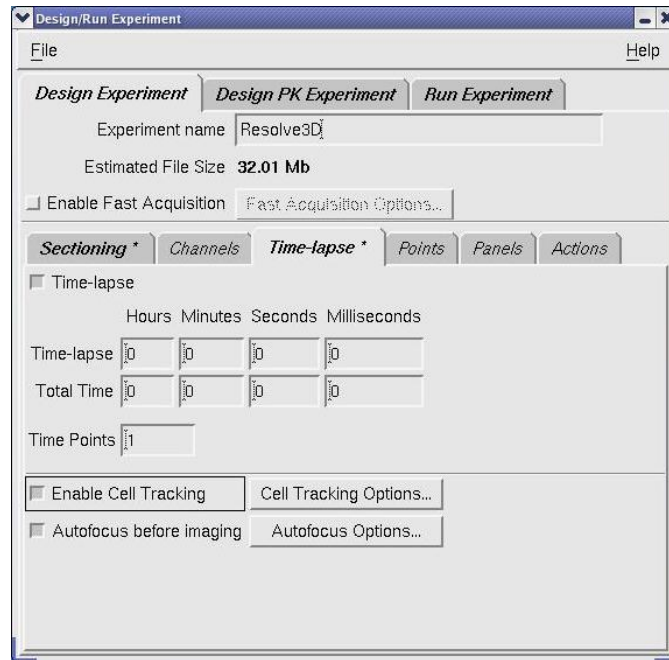
After you specify an ROI around an object of interest, the software determines the center of the cell and establishes a recognizable pattern within the ROI. In subsequent images, the software recognizes this pattern and recalculates the center of the cell on-the-fly. The position of the new center is compared with the position of the previous center and, if the cell has moved beyond a specified threshold, the system automatically moves the stage and re-centers the cell in the field of view.



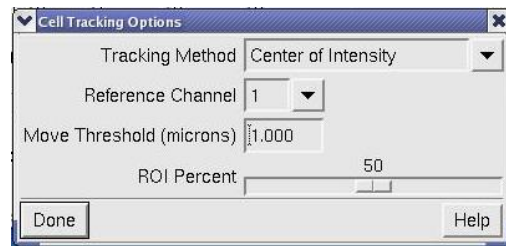
At Time Points 1 and 2, the cell is moving through the ROI (the inner square), but the center is still within the Move Threshold (the circle). At Time Point 3, once the center of the cell touches the threshold boundary, the stage moves to re-center the cell within the ROI. Note the new stage position displayed in the lower-right corner of Time Point 4.

To use Cell Tracking:

1. Set up a time-lapse experiment as shown in *Setting up Time-Lapse Experiments* on Page 40.
2. On the Time-lapse tab on the Design/Run Experiment window, select the **Enable Cell Tracking** option.



3. Click **Cell Tracking Options**.



4. Manually acquire an image for each channel and determine which single channel best identifies the features of your specimen. In the **Reference Channel** list, specify that channel. (The channels are numbered in the order that they are listed on the Design Experiment Channels tab.)



Note *DeltaVision* uses the reference channel for image recognition.

5. In the **Move Threshold** field, enter the distance in microns that the cell must move to trigger stage movement.
6. Specify the area around the cell (the region of interest or ROI) that you want *DeltaVision* to use for image recognition. For more about this option, see *ROI Percent* on Page 68.
7. If you are performing an experiment on a single cell, use the stage controls to center the cell (laterally) in the field of view. If you are performing a point

visiting experiment, make sure that the points that you are monitoring are centered before you start your experiment.

8. Click the Run Experiment tab and then click **Start Scan** to run the experiment.

Guidelines for specifying Cell Tracking Options

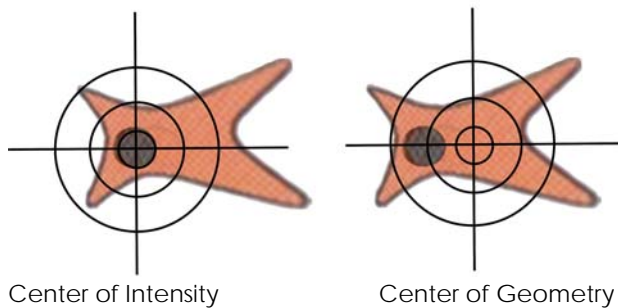
You can specify several parameters that *DeltaVision* uses to track cells:

- Tracking Method
- Reference Channel
- Move Threshold
- ROI Percent

Tracking Method

The tracking method is the method that *DeltaVision* uses to determine the center of the feature. This center is recalculated after each image is acquired. You can choose from two tracking methods:

- Center of Intensity calculates the center of the feature based on intensity values.
- Center of Geometry calculates the center of the feature based on its geometry.



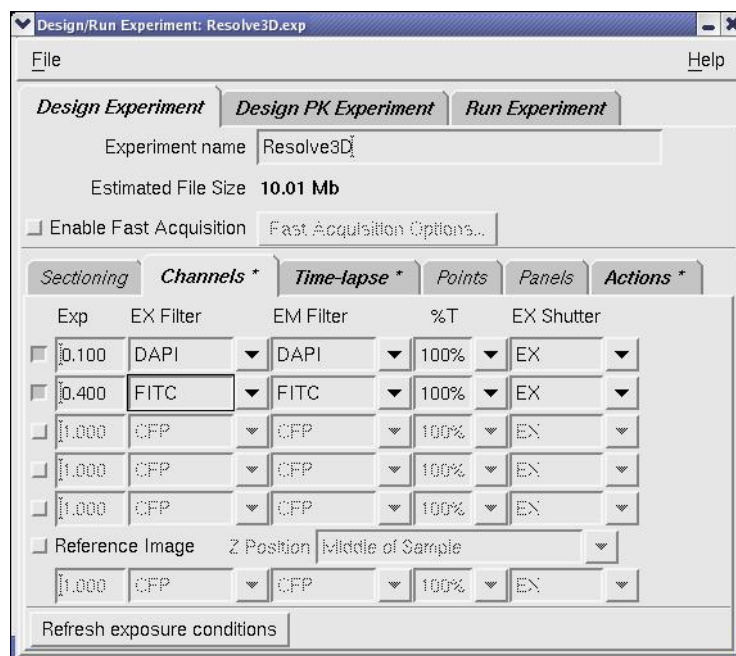
Reference Channel

DeltaVision uses the Reference Channel for pattern recognition. If you are acquiring more than one channel, choose the channel that has the most distinctive features.



Note The term, *Reference Channel* should not be confused with *Reference Image*, which is something very different. Reference images are described later in this chapter in *Acquiring a Reference Image*.

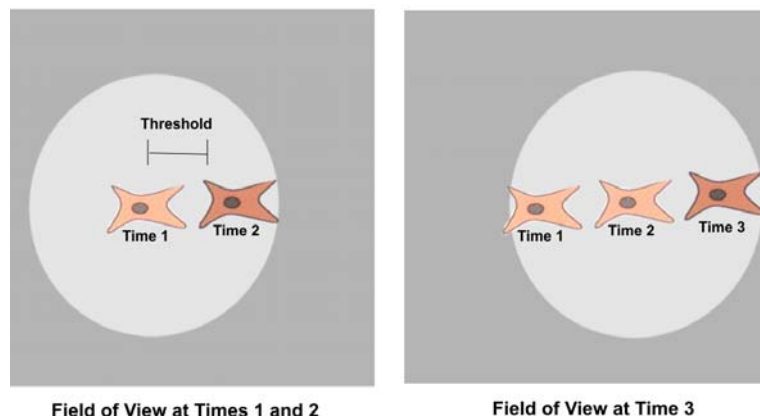
Channels are numbered as they appear in the Experiment Designer window. In the following example, DAPI is Channel 1 and FITC is Channel 2.



Move Threshold

The Move Threshold is the distance the center of the cell (as defined by the tracking method) must travel before the system resets the stage. When the cell moves beyond this threshold, the stage moves so that the center of the cell retains its original position in relationship to the center of the field of view.

Live cells, by their very nature, are constantly on the move. When choosing a Move Threshold, it is important to select one that is small enough to keep the cell in the field of view, yet large enough to buffer out needless stage movement that could result from numerous small cell movements.



Field of View at Times 1 and 2

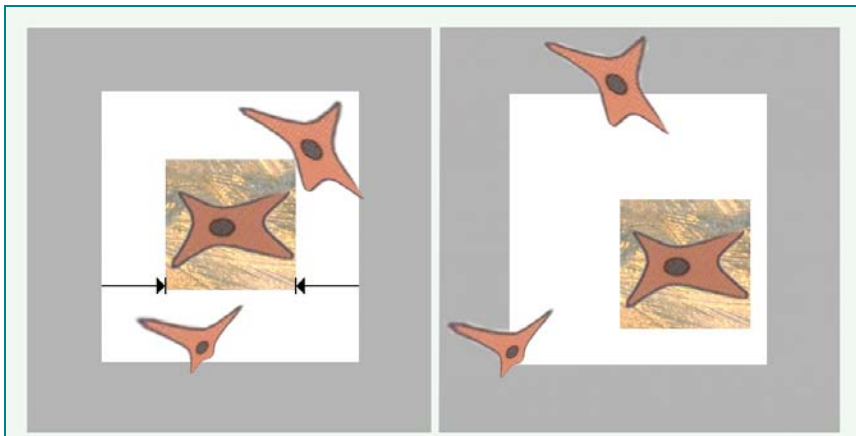
Field of View at Time 3

Cell tracking lags cell movement. Cell movement exceeds the threshold between Time Points 1 and 2 (left). In response, *DeltaVision* moves the stage so that it is centered on the cell position at Time Point 2. The stage is at this position when Time Point 3 is acquired (right).


ROI Percent

With rare exceptions, few live cells are isolated on the substrate. Most are genetically “programmed” to seek attachment and communication with other cells. When performing live cell microscopy, not only is it important to filter out unnecessary stage movement, it is also important to define an appropriate region of interest within the field of view. This region of interest must be large enough to include the entire cell or structure of interest, yet small enough to exclude other cells or structures that may tend to wander in and out of the field of view throughout the experiment.

The ROI Percent parameter defines how much of the field of view the software will use for image recognition. In the software, ROI units are specified as a percentage of the width of the field of view. For example, a 50% ROI has a width that is 50% of the width of the field of view.

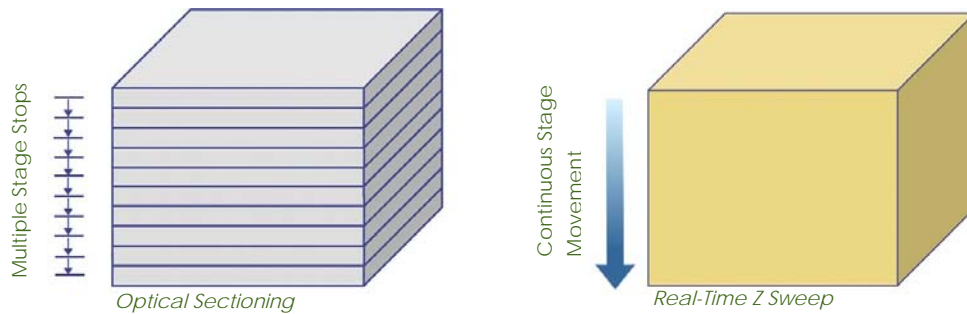


In the first image, the width of the ROI is defined as 50% of the width of the field of view. In the second image, the stage has moved to keep the cell of interest within the ROI. Perimeter cells have also moved, almost entirely out of the field.

 **Tip** If *DeltaVision* loses the cell because it moves out of the field-of-view, you can update the point coordinates by manually finding the cell and centering it in the field-of-view. The point list is automatically updated.

Acquiring 3D Z Projections with OAI

Optical Axis Integration (or OAI), also referred to as Continuous Z Sweep, is useful for acquiring 3D Z projections of live specimens. Instead of collecting an individual image at each focal plane, OAI collects and integrates one continuous image through an extended Z movement.



Instead of acquiring multiple images, Real-Time Z Sweep acquires one image during a continuous stage movement and instantly creates a 3D Z Projection.

Real-Time Z Sweep has significant advantages for applications such as Leading Edge Motion Analysis, Fast Organelle Dynamics, Microtubule Dynamics, and Fluorescence in situ Hybridization (FISH). It is especially useful for studies of objects that are moving in 3D space (e.g., kinetichores in a cell nucleus or other rapidly moving structures).

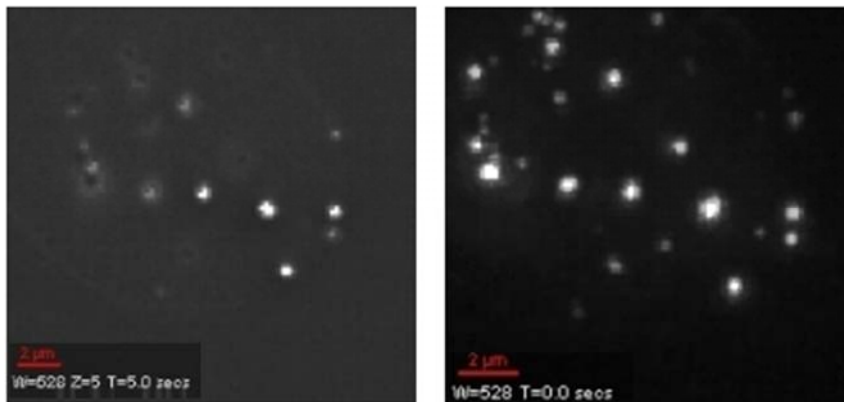
Continuous Z Sweep versus Traditional Projections

If you are acquiring data objects that you plan to use for 2D projections, Continuous Z Sweep provides several advantages over creating 2D projections from multiple optical sections:

- Complete Z data acquisition collects all data in the interval of interest (with 2D imaging or Z section sampling, some data is lost.)
- Fast data acquisition provides accurate image registration of rapidly moving objects.
- Low total exposure time reduces the risk of damage to the specimen.
- Low total read noise (the camera is only read once) improves signal-to-noise ratio.

Note For samples that contain a large amount of fluorescence throughout 3D space (for example, a tumor spheroid that has a lot of fluorescence), optical sectioning may provide better results than OAI.



The following 2D image of endosomes in a HeLa cell (left) and a 3D Z projection (right) of the same area were acquired under similar conditions. The additional data in the 3D Z projection include objects that moved out of the depth-of-field of the 2D image during the data acquisition process.

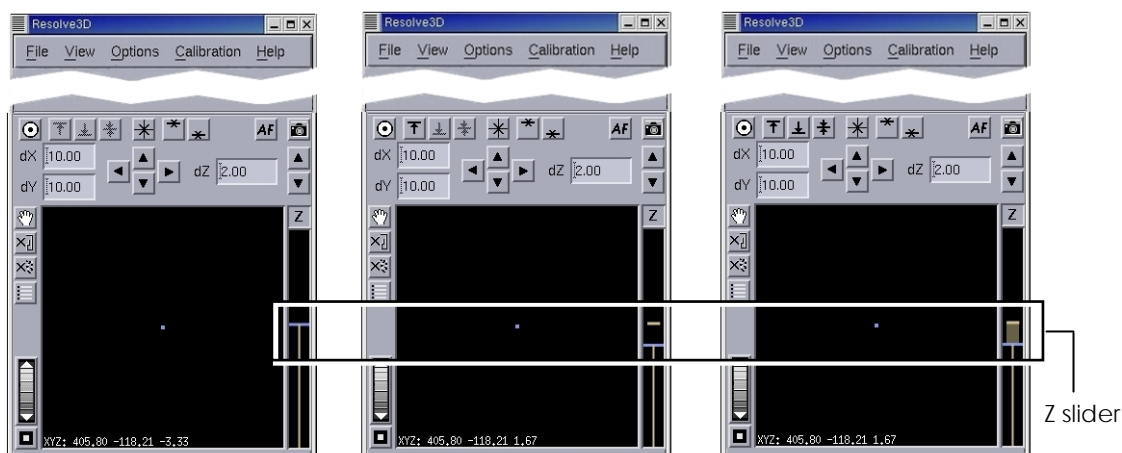


endosomes in a HeLa cell (left) and an instant 3D Z projection (right) of the same area

Using Continuous Z Sweep

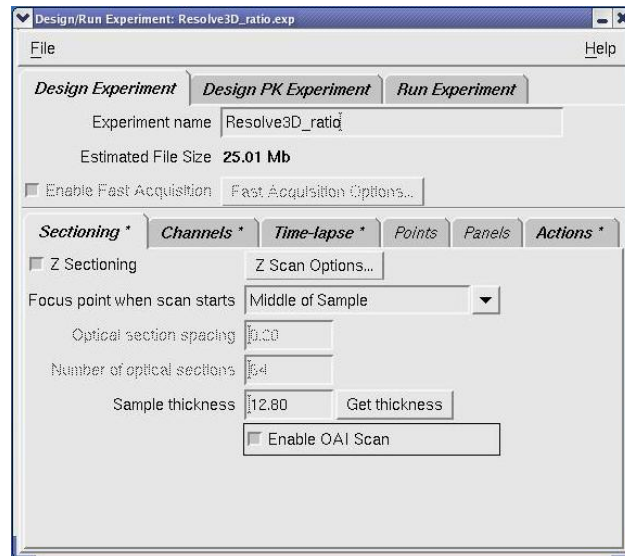
To set up a Continuous Z Sweep Experiment:

1. Set up your experiment as shown in *Creating and Running an Experiment Macro* on Page 31.
2. In the Resolve3D window, use the Z Slider on the right side of the Resolve3D window to find and center the object of interest. Drag the slider up to find the top of your sample. (When you release the mouse button, an image is acquired and displayed in the Data Collection window.) Drag the slider and acquire images until you are satisfied. Then press the  button to mark that location. Next, use the slider to find the bottom of the sample. When you are satisfied, press the  button to mark that location.



Use the Z slider to find the top and bottom of a sample. The sample thickness is indicated by the wide line on the Z slider (shown on the right image).

3. Click **Experiment** to open the Design/Run Experiment window. Then click the Sectioning tab.

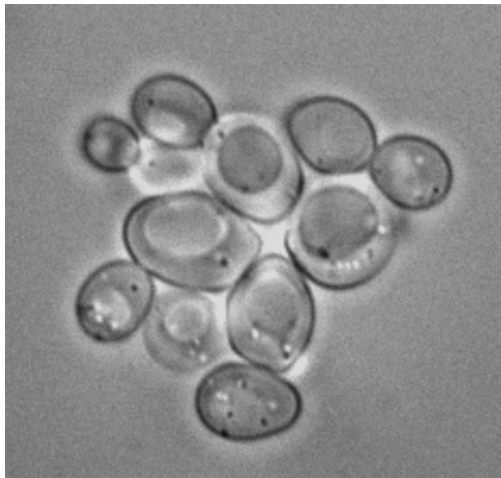


4. Select the **Z Sectioning** and **Enable OAI Scan** options.
5. Click the Run Experiment tab and click **Start Scan** to start the experiment.

Note When deconvolving OAI images, select the **More Options** button on the Deconvolve window, and then select the **Deconvolve Projections** option in the **More Deconvolution Options** dialog box.

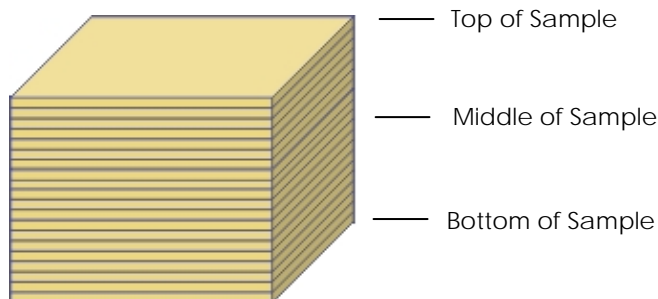
Acquiring a Reference Image

You can use an alternate filter or the transmitted light to acquire a reference image that can be combined with other images. This option is useful for Differential Interference Contrast (DIC) analysis. It can also be useful for other types of reference images.



A reference image of yeast cells

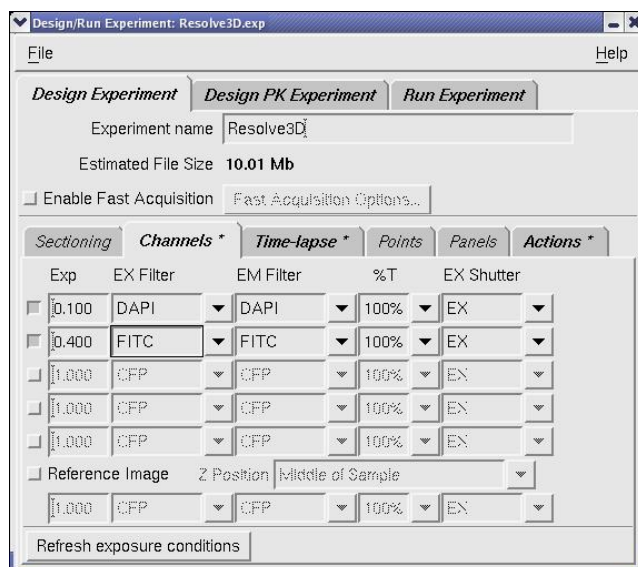
You can specify to acquire the reference image at the top, middle, or bottom of the sample.



Note It is important to optimize the reference image settings prior to defining them in the Experiment Designer.

To create a reference image:


1. Set up a 3D Sectioning experiment (see *Sectioning Specimens for 3D Images* on Page 33).
2. In the Design/Run Experiment window, click the Channels tab and select the channels for your experiment (see *Selecting Filters* on Page 40).
3. Select the **Reference Image** option.
3. In the Z position list, choose whether to acquire the reference image at the top, middle, or bottom of the sample.
4. Under the Reference Image, select which filters to use.
5. In the EX Shutter field, select the type of light to use for the image. EX is the Excitation (or the arc lamp) and TRANS is the transmitted light.



This experiment specifies using the DAPI and FITC filters to acquire images of each section and using the transmitted light to acquire a reference image in the middle of the sample.

6. Save and Run the Experiment.

After *DeltaVision* acquires the data, it creates two image files. One file contains the reference image data and the other file contains all of the other data.

 **Tip** After you acquire the images, you can use the Image Fusion tool in *softWoRx* to combine the reference images with the rest of the images.

6

Data Collection Techniques

This chapter provides guidance and suggestions for:

- Finding a Specimen and Recording Its Position
- Finding Exposure Time
- Using Köhler and Critical Illumination
- Monitoring Data Acquisition
- Editing Experiment Macros

Finding a Specimen and Recording its Position

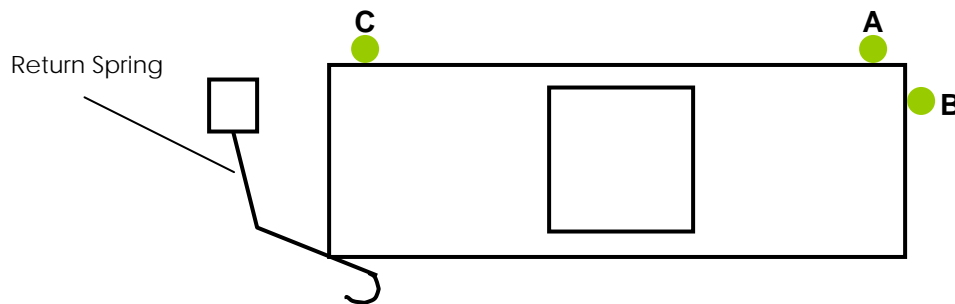
Recording the position of your slide is useful when you are conducting a point visiting experiment and you need to remove the slide before you are finished. Use the following instructions to find a sample and record its position on the slide with the Repeatable Slide Holder. When you resume your experiment, you can place the slide in the position that you recorded.

Note If you are using an oil immersion lens, you must apply immersion oil to the sample. See *Appendix A: The Oil Immersion Kit*, for more information about the oil calculator.

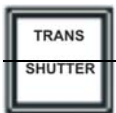
To find and center the specimen:

1. Choose the objective by rotating the objective turret. Be sure to select the same objective in the Resolve3D window.
2. Secure the slide. Since *DeltaVision* is an inverted microscope, the sample must be placed with the cover slip facing down toward the objective. If you are using a standard microscope slide (1" x 3" or 25 mm x 75 mm), you can use the Repeatabile Slide Holder to hold the slide. To do this, pull the return spring to the left and place the Slide Cover Slip down onto the Slide Holder so that the upper right corner is pressed laterally against the brass locator (shown as A and B in Figure 5) and the upper left edge is pressed laterally against the brass locator C. Then gently release the return spring to secure the slide. Push down on the slide to make sure that it is fully seated in the holder.

Figure 5: The Repeatabile Slide Holder



3. Choose the desired eyepiece (EP) filter.
4. Select the desired excitation (EX), neutral density (%T), and emission (EM) filters. (These parameters should automatically be in place after selecting the eyepiece filter, unless non-standard arrangements are needed.)
5. Rotate the Beam Selector at the base of the microscope to direct the light collected by the objective to the eyepiece.
6. To use the transmitted light to locate the specimen, open the transmitted light shutter by pressing TRANS SHUTTER on the keypad. This allows white light to transilluminate the sample. (The intensity for the transmitted light is controlled using the %T field on the Resolve3D menu.)
7. Set the stage speed using the keypad SLOW, MEDIUM, or FAST Buttons. Medium is typically the best speed to start with. (These buttons control the stage speed when you are using the joystick or keypad to move the stage.)



8. Open the EX shutter and find the desired focal plane optically using either the focus knobs on the microscope or the Resolve3D Z stage controls.



Note If you are using an Olympus or Nikon microscope, rotate the top of the focus knob toward you to move the objective up and away from you to move it down (when you are sitting in front of the microscope).

9. Use one of the following methods to maneuver the slide and find an area of interest:

Keypad

The buttons on the keypad are used for movement in the X, Y, and Z directions. Using the arrow keys on the keypad causes the stage to move by steps. The size of each step is doubled each time the STEP INCREASE button is pressed and halved each time the STEP DECREASE button is pressed. By adjusting the step size to the frame size, you can create a condition where each press of a step arrow will move the sample one frame. This is a convenient way to scan a large area for rare events (e.g., mitosis). Since the step movements are rapid, using the arrow keys in this way can be much less fatiguing than using the joystick.

Joystick

The joystick controls stage movement in the X and Y directions.

Workstation

The Resolve3D module allows you to finely control stage motion. The arrow buttons move the stage in discrete increments that are indicated by the values in the **dX**, **dY**, and **dZ** text boxes. Resolve3D provides the only mechanism from which to obtain discrete step sizes.

Focus Knob

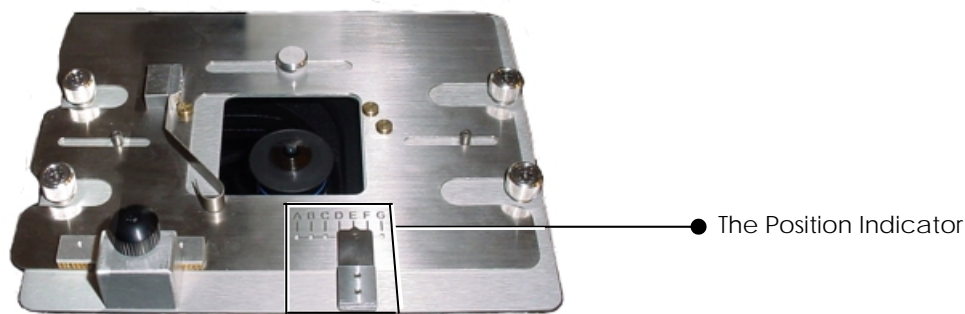
The manual focus knob on the microscope base moves the nosepiece/objective in the Z direction.



CAUTION: To record and maintain accurate stage coordinates, focus by moving the stage with the Resolve3D controls instead of using the focus knobs to move the objective. Resolve3D cannot track the movement of the objective.

10. Tighten the Focus Lock on the left side of the focusing knob (see Page 13).
11. Use the keypad to find the approximate center of the specimen and place it in the center of the field of view using stage controls.
12. Direct light to the camera port using the Beam Selector knob on the microscope base.

13. Record the position of the slide on the Position Indicator (the letter scale at the bottom of the Repeatable Slide Holder).



Finding Exposure Time

The exposure time correlates with the signal intensity level for the image acquisition. Finding the appropriate exposure time for each filter in an experiment is crucial to acquiring the best image data. Many factors must be considered. Although you want to see the maximum intensity in each wavelength, you must not saturate the camera or photo-bleach your sample.

High-Speed CCD Camera saturation occurs when the intensity values reach 4145 counts. The 12-bit High-Speed camera has an intensity range of 0 to 4095 counts. (The EM CCD 16-bit High-Speed camera has an intensity range of 0 to 65535 counts.) For successful deconvolution, a minimum intensity of 50 counts above background is recommended.

Note If you are using 0.5X Gain, the saturation is less than 4095 counts. For the EM CCD camera (a 16-bit camera), saturation is over 50,000 counts.

Photo-bleaching occurs when the fluorescent dyes lose their emission intensity as they are exposed to illumination. Certain dyes are more susceptible to photo-bleaching than others. The potential for excessive photo-bleaching is increased with increased exposure time and decreased by use of a higher value neutral density filter.

There are two ways to decrease illumination, and therefore the likelihood of photo-bleaching:



- Decrease exposure time.
- Increase neutral density value.

Note For live cell imaging, cells tolerate shorter exposures better than long exposures, even at increased illumination intensity.

The response of increasing the exposure time is roughly linear. Therefore, if an exposure time of 0.2 sec results in a maximum intensity level of 1000 counts, then an exposure time of 0.4 sec will result in a maximum intensity level of approximately 2000 counts.

Use the following instructions to find the best exposure time for each excitation wavelength. This procedure must be performed for each excitation wavelength used in the experiment.

To find exposure time:

1. Set the neutral density filter to 100%.
2. Set the exposure time to a low value (e.g., 0.1 sec).
3. Set **dZ** to the desired value to sample different images throughout the sample.
4. Click the **dZ** textbox.
5. Enter the desired value for the Z step.
6. Move through the area of interest by pressing  and  in the Stage dialog to move the stage in the Z direction.
7. Click **Acquire**. If performing a 2D experiment, go to Step 13.
8. Continue performing steps 6 and 7 to obtain a sampling of Z sections. Find the maximum intensity in these sample images by observing the value of the **Max** field in the Resolve3D window. The focal plane with the highest maximum intensity value is the plane of optimal focus.



Note There could be a higher intensity value in the image data that you did not sample. Therefore, do not set the exposure time to the limits of the sampled data because a higher intensity, unsampled plane may saturate the camera.

9. Move to the plane of optimal focus. This is the Z section with the highest intensity value.
10. From the Resolve3D menu, choose **View | Point List**.
11. Click **Mark Point**. The Z value is the third coordinate listed for the point.
12. Document the Z value with the brightest intensity.
13. Increase exposure time, but consider the amount of exposure the specimen can tolerate. Intensity values increase in direct proportion to exposure time (e.g., doubling the exposure time doubles the intensity values).
14. Repeat for all desired wavelengths.

Using Köhler and Critical Illumination

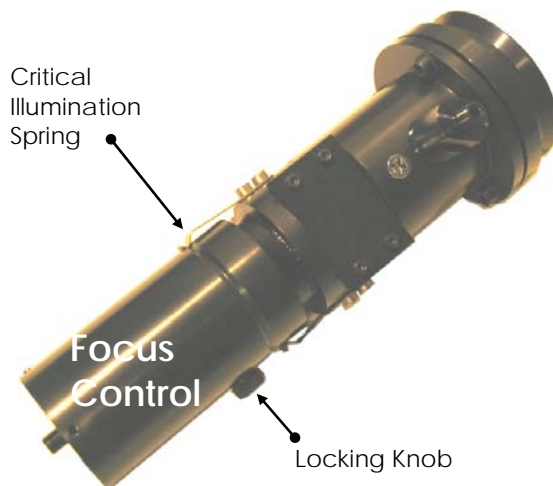
DeltaVision Core allows you to easily switch between two types of illumination:

- Köhler Illumination is the most commonly used form of illumination. It provides very even specimen illumination across the field-of-view. The light uniformly drops off as the distance from the focal plane increases.
- Critical Illumination directs the entire light source to the size of the detected area, and not the entire sample. With Critical Illumination, more light is directed to the focal plane and the out-of-focus light drops off more rapidly than in Köhler Illumination, based on the size of the field-of-view. Critical illumination also provides better axial (Z) and lateral (X,Y) contrast.

You will typically use Köhler Illumination for most of your data collection. However, Köhler Illumination can be insufficient for faint signals. For low abundant probes that require more light and better contrast, use Critical Illumination.

To switch to Critical Illumination:

- Loosen the Locking Knob and pull the Focus Control back (away from the stand) until it snaps into place in the Critical Illumination position. Then tighten the Locking Knob.



In Critical Illumination position, the Focus Control is extended so that the Critical Spring is in the groove.



CAUTION: When you are adjusting the Fiber Optic Module, be careful not to disconnect the Fiber Optic cable or to bend it in a diameter that is less than 24".

To switch to Köhler Illumination:

- Loosen the Locking Knob and push the Focus Control toward the stand until it snaps into place in the Köhler Illumination position. Then tighten the Locking Knob.



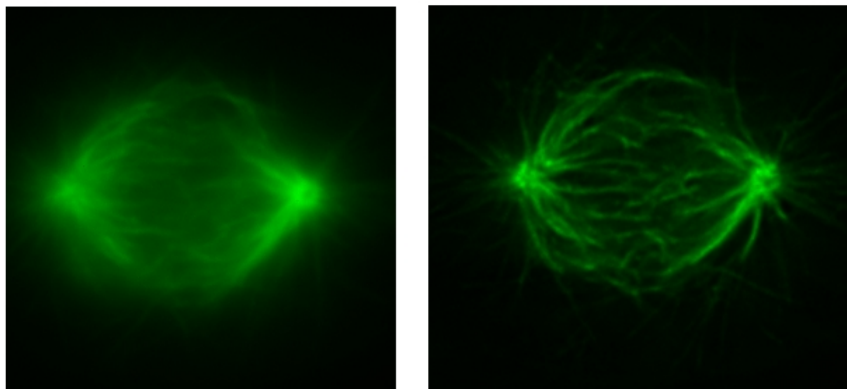
In the Köhler Illumination position, the Fiber Optic Module is pushed in so that the Köhler Illumination Spring is in the groove.

Monitoring Data Acquisition

You can set options that control how images are displayed as they are acquired.

Viewing Deconvolved Image Previews

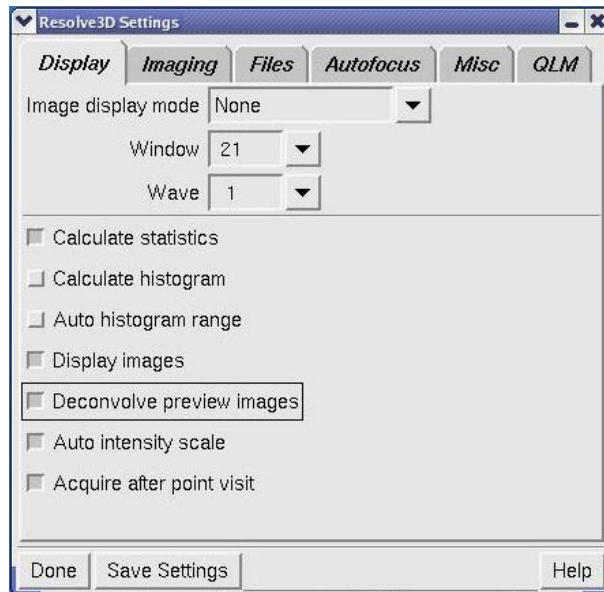
You can use 2D deconvolution to get a more accurate representation of what data will look like after deconvolution.




These images show how images are displayed during data acquisition, with and without Real-Time 2D Deconvolution.

To view previews of deconvolved images:

1. From the Resolve3D window, click **Settings** to open the Resolve3D Settings dialog box. Then click the Display tab.

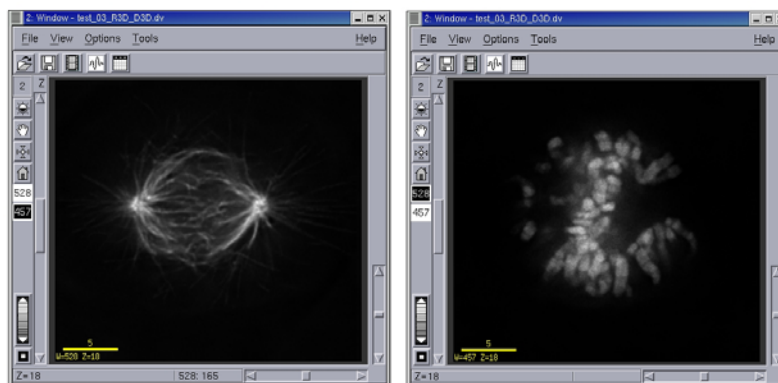


2. Select the **Deconvolve preview images** option.

 **Note** This option does not provide a full iterative deconvolution, but it allows you to preview images as you collect them.

Selecting Viewing Modes

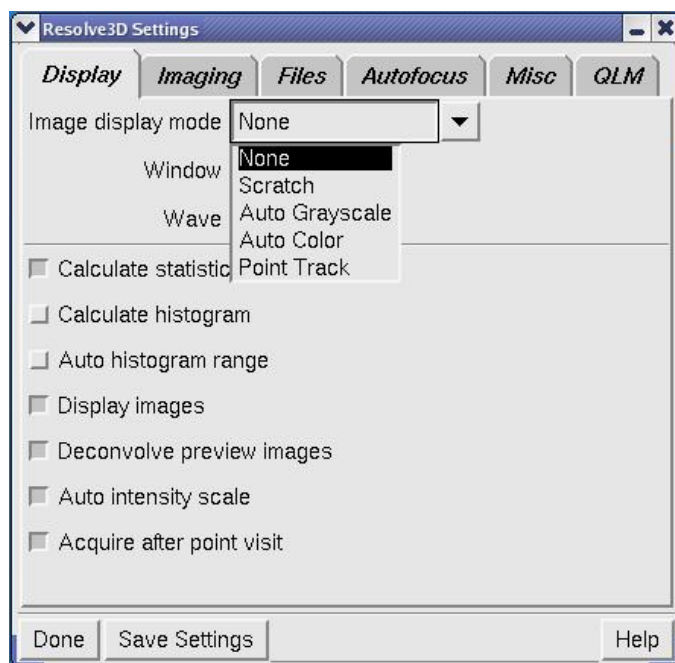
You can select from several modes for displaying images in the Data Collection window (or windows) as they are collected. You can choose other modes to display images in color, display each point in a point visiting experiment in a separate window, or to display each channel in a separate window, as follows.



Auto Grayscale mode displays a separate window for each channel.

To select the display mode:

1. From the Resolve3D window, click **Settings** to open the Resolve3D Settings dialog box. Then click the Display tab.
2. On the **Image display mode** list, select a display mode.

**Table 1: Image display modes**

Mode	Description
None	Displays images in the current window.
Scratch	Displays all images in the default Data Collection window (Window 21).
Auto Grayscale	Displays images in a separate window for each emission filter.
Auto Color	Displays images in color as they are collected. When using Point Visiting, images are automatically displayed in separate windows for each point. This option should be used only when you are running an experiment.
Point Track	Opens a separate window for each visited point in a point-visiting experiment.

Displaying Statistics and the Histogram

You can choose to display statistics and a histogram of the intensity values of each image as it is collected. These values are displayed at the bottom of the Resolve3D window. The Min, Max, and Mean values are the minimum, maximum, and mean intensity values. The histogram displays the intensity distribution.

To display statistics and the histogram:

1. From the Resolve3D window, click **Settings** to open the Resolve3D Settings dialog box.
2. Click the Display tab. Then select from the following options.

To	Select
Calculate image intensity statistics	Calculate Statistics. Note: You can improve readout speed by disabling this option.
Calculate and display an image intensity histogram	Calculate histogram
Automatically scale the histogram width for each image that is analyzed.	Auto Histogram Range Note: This option changes only the display of the histogram. It does not change the image data.

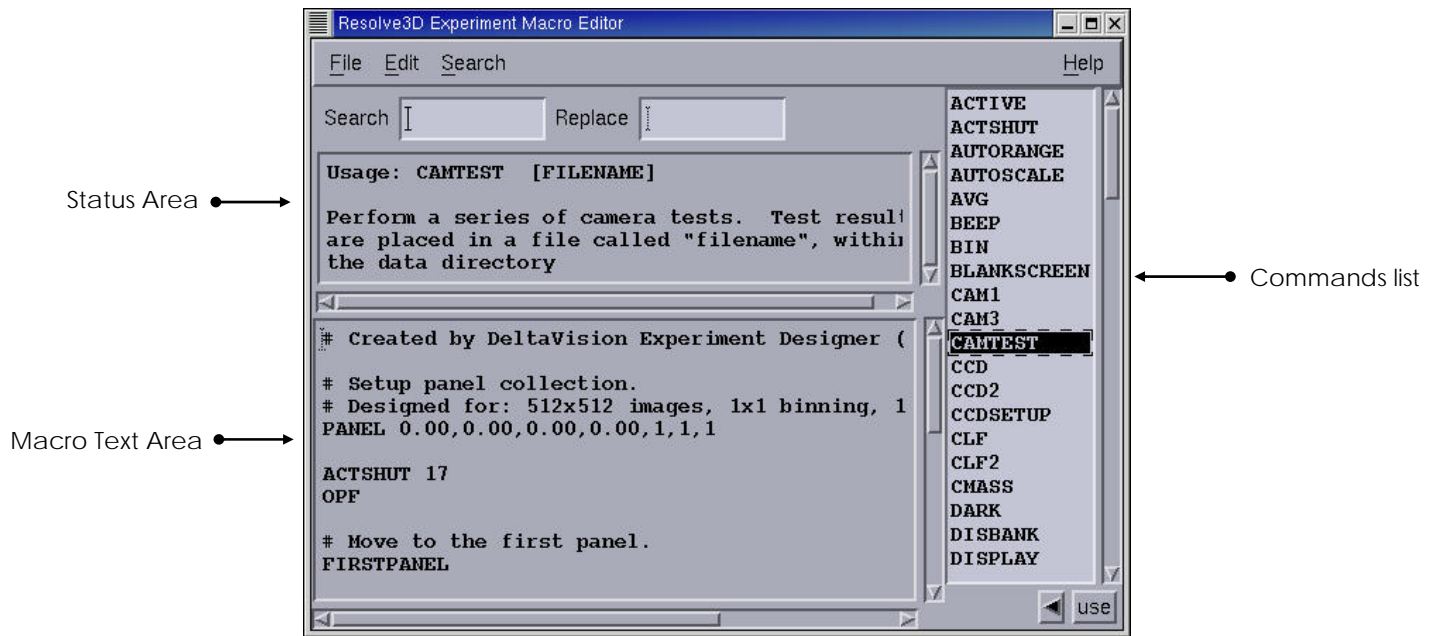
Editing Experiment Macros

The Experiment Macro Editor is used to create or edit Resolve3D experiment macros (command scripts) that control the *DeltaVision* microscope. Most experiment macros can be generated using the Design/Run Experiment window, but you may need to create custom macros for certain types of experiments.

The best way to get started is to modify an existing macro with the Experiment Macro Editor. One approach is to use the Design/Run Experiment window to generate a macro and then edit it. Another approach is to use a reference macro.

To open the Experiment Macro Editor:

1. On the Resolve3D window, click **Experiment**.
2. In the Design/Run Experiment dialog, choose **File | Edit** to open the Experiment Macro Editor.



The Experiment Macro Editor

Menu Bar

The menu bar **File** menu contains commands to open and save experiment macro files. The **Save & Quit** option saves the macro file with the current name. The **Edit** menu has commands for manipulating text. The **Search** menu provides search and replace capabilities and the **Help** menu displays additional information.

Search & Replace

These text fields are used to specify a search and replace pattern. (You will need to enter strings in these fields before you use the **Search | Replace Text** menu command.)

Status Area

The text area below the **Search** and **Replace** fields is used by the Macro Editor to supply you with status and hint information.

The Macro Text Area

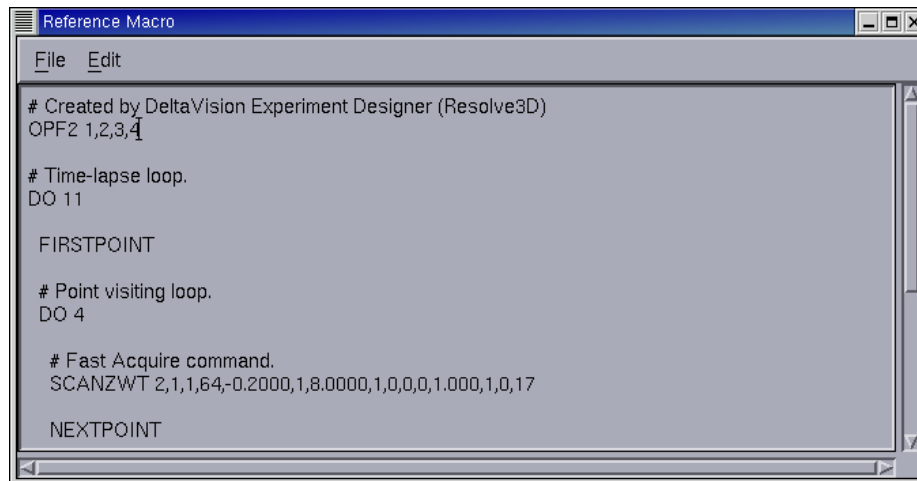
The Macro Text Area below the Status Area is the main working area of the Macro Editor.

Commands List

This is the list of available commands. When you highlight a command in the list by clicking it, you will be provided with information about the command in the Status Area. If you double-click or select the **Use Item** button below this list, the command is inserted in the Macro Text Area at the current cursor location.

To edit a reference macro:

1. On the Resolve3D Experiment Macro Editor window, choose **File | Open Reference Macro**.
2. In the Open Experiment Macro File dialog box, select a macro file and click **OK** to open it.



3. Use the mouse to select the part of the reference macro that you want to copy.
4. On the Reference Macro menu, choose **Edit | Copy**.
5. On the Resolve3D Experiment Macro Editor, choose **Edit | Paste** to copy the macro into the macro editor.
6. Edit and save the file as a new experiment macro.

Facility Requirements & Components

This chapter describes the main components of the *DeltaVision* System. It includes the following sections:

- *Electrical and Environmental Requirements* describes the *DeltaVision* operating and facility requirements.
- *Overview of Components* shows the location of the key system components.
- *Optical Components* describes the system light sources, cameras, and filters.
- *Desktop Components* provides information about the monitor, the keypad and joystick, the vibration isolation table that supports the microscope, and other components.
- *Cabinet Components* describes the combined Instrument Controller and Microscope Interface Chassis (IC/MIC) and the Workstation.
- *Other Standard Components* describes the Repeatable Slide Holder, the Fiber Optic Module, the tool kit, and standard software.
- *Optional Components* describes the Quantifiable Laser Module (QLM), the EM CCD camera, The *Rainbow* option (dual light sources for DIC), and the *softWoRx Explorer* application.
- *Consumable Parts* lists the fuses and other components that you will need to replace to maintain the system.

Electrical and Environmental Requirements

An important aspect of collecting high quality images is meeting the proper electrical and environmental requirements for the system.

Electrical Requirements

Line Requirements

Operating Frequency: 50/60 Hz

Operating Power: 100-127 VAC requires 8A
200-240 VAC requires 4A

Transients: Transient over-voltages in accordance with Installation Category II in IEC 664

Maximum Power: 1200 VA

Power Cord Set Requirements

The power cord set received with *DeltaVision* meets the requirements for use in the country where you purchased the equipment.

General Requirements

The requirements listed below are applicable to all countries:

- The length of the power cord set can be a maximum of 9.75 feet (3.0 m).
- All power cord sets must be approved by an acceptable accredited agency responsible for evaluation in the country where the power cord set will be used.
- The power cord set must have a minimum current capacity of 10A for 230 VAC systems or 15A for 100-120 VAC systems, as required by each country's power system.
- The appliance coupler must meet the mechanical configuration of an EN60320/IEC 320 Standard Sheet C13 connector, for mating with the appliance inlet on the Isolation Transformer Assembly.

Country-specific Requirements

The following table shows the accredited agency and power cord set requirements for each country.

Country	Accredited Agency	Power Cord Set Requirements
Australia	EANSW	The flexible cord must be <HAR> Type HO5VV-F, 3-conductor, 1.0 mm ² conductor size. Power cord set fittings (appliance coupler and wall plug) must bear the certification mark of the agency responsible for evaluation in the country where it will be used.
Austria	OVE	
Belgium	CEBC	
Denmark	DEMKO	
Finland	SETI	
France	UTE	
Germany	VDE	
Italy	IMQ	
Norway	NEMKO	
Sweden	SEMKO	
Switzerland	SEV	
United Kingdom	BSI	
United States	UL	The flexible cord must be Type SJT or equivalent, No. 14 AWG, 3-conductor. The wall plug must be a two-pole grounding type with a NEMA 5-15P (15A, 125V) configuration.
Canada	CSA	
Japan	JIS	The appliance coupler, flexible cord, and wall plug must bear a "T" mark and registration number in accordance with the Japanese Dentori Law. The flexible cord must be Type VCTF, 3-conductor, 2.00 mm ² conductor size.

Environmental Requirements

An important aspect of collecting high quality images is having the proper environment for the system. The following important environmental requirements are outlined in this section.

General Environmental Requirements

Floor space:

DeltaVision Core - 3 ft × 6 ft (90 cm × 180 cm). Include 18 in (45 cm) space behind instrument rack.

personalDV - 22in x 54 to 62in (depending on whether or not the keyboard is kept in a slide-out tray). Include 18 in (45 cm) space behind instrument rack.

Maximum System Weight: 940 lbs (425 kg).

Service: Indoor use only.

Temperature: 65 - 77 °F (18 - 25 °C), daily variation of no more than 3 °F (1.8 °C). The actual room temperature should be stable to within 1 degree (Fahrenheit or Celsius) per hour. Fluctuations in temperature will affect microscope optics, which can cause the specimen to drift approximately 1 µm per 0.1 degree Celsius.

Humidity: Stable humidity levels under 50%, with daily variations of less than 10%. High humidity can result in condensation on the CCD camera window that obscures image formation. Excessive humidity may also reduce filter life and may result in chromatic aberrations in the images.

Altitude: up to 6550 ft (2000 m).

Pollution: POLLUTION DEGREE 2¹ in accordance with IEC 664.

Ingress Protection Level: IP20

Communication Recommendations

Connect the workstation to a local area network for data storage. To connect to a network, you will need an IP address, domain name server address, and network mask. A connection to the Internet will provide access to Applied Precision's web site.



CAUTION: Applied Precision, LLC is not responsible for damage or harm to the workstation or scanner due to network security breaches.

Having a telephone in the same room as the system will facilitate communication with Technical Support.

Air Movement

Air movement around the microscope can cause specimen drift on the scale of several microns. Two common sources of air movement are window air conditioners and open windows. Central air conditioning is recommended. However, the system should not be placed in the direct path of the incoming air.

Vibration Isolation

The vibration absorbing design of the system minimizes motion artifacts from internal vibration due to shutters, filter wheels, and stage movement. The system is also designed to damp out external vibration as well. Avoiding locations near refrigerators, elevators, ventilation equipment, and other sources of vibration will improve image resolution.

¹ IEC 61010-1; 2nd ed. International Electrical Commission defines POLLUTION DEGREE 2 as follows: "Normally only non-conductive POLLUTION occurs. Occasionally, however, a temporary conductivity caused by condensation must be expected."

Ambient Illumination

For best results, minimize ambient illumination during data collection. A light-tight room is recommended. Ensure that there are no light sources pointed downward into the lens.

A small desk lamp located near the workstation is recommended for preparing and monitoring experiments.

You can press the BLANK SCREEN key on the keypad to darken the monitor for improved image quality. Pressing any key on the keyboard restores monitor function.

Dust

It is important to minimize dust on the microscope components because dust on components can cause spots on microscope images. Minimize contamination by maintaining a clean room and covering the microscope when it is not in use.

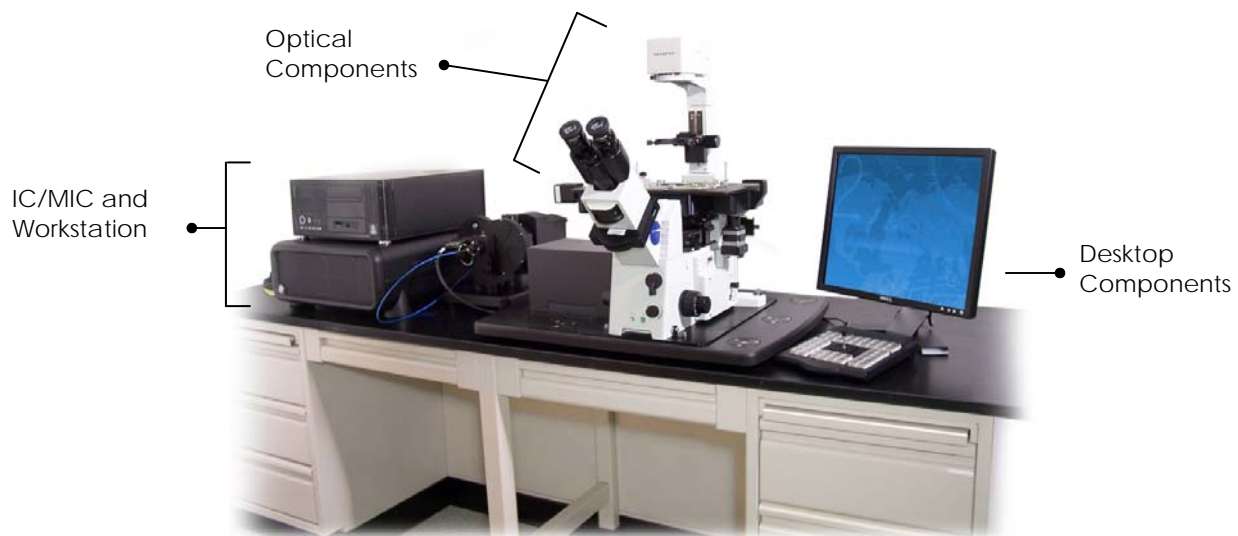
Overview of Components

The main standard *DeltaVision* components for both *DeltaVision Core* and *personalDV* are shown below. Detailed descriptions of these components and descriptions of optional components are included in the following sections.

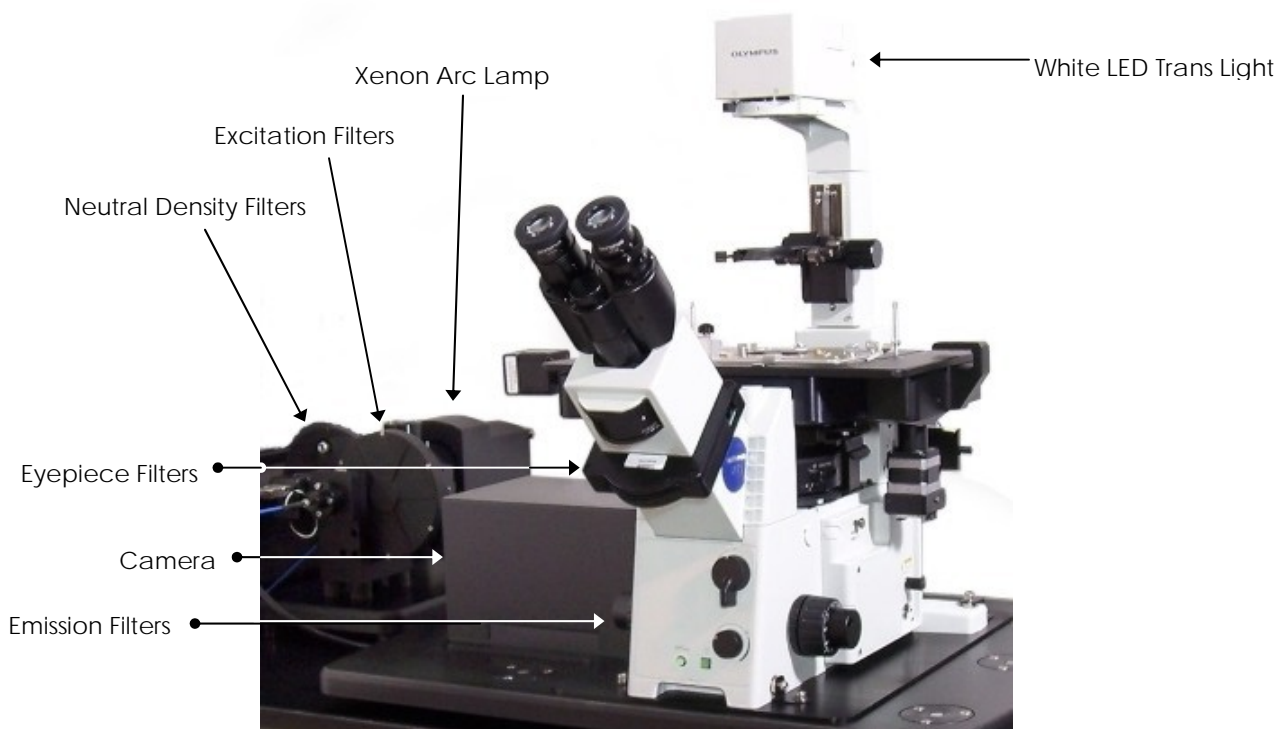
Standard DeltaVision Core Components



personalDV Components



Optical Components



Note Optical components are shown for a typical installation, which includes the Olympus IX71 Microscope. Other microscope configurations vary slightly. The Olympus IX71 is currently the only microscope available.

Fluorescence Microscope

The microscope is an advanced research grade epifluorescence inverted microscope. Each objective is qualified by Applied Precision to guarantee the highest possible quality. The point spread function (PSF) is measured in order to uphold the image quality. The microscope supports a Differential Interference Contrast (DIC) module (this is an optional component). A transmitted light shutter is included with your system to enable automated DIC and Brightfield image acquisition.

Optical Filters

The system uses a four-band polychroic beam splitter and filter wheels rather than a simple dichroic filter cube. The excitation, neutral density, and emission filters are selected in one of three ways:

- Selecting options in the Resolve3D acquisition software
- Selecting a mode from the keypad
- Rotating the eyepiece filter wheel

Note The eyepiece filter wheel is operated manually but reports its position to the instrument controller, which in turn adjusts the excitation and emission filters.

Depending upon the environment, filters last between one and three years. To ensure optimal performance, filters should be periodically inspected and replaced if necessary. Consult *Chapter 9: Maintenance* for a detailed explanation of this procedure.

The following standard *DeltaVision* Filter set is included in all *DeltaVision Core* systems.

Table 2: *DeltaVision* Standard Filter Set

Filter Name	Excitation CWL/BP	Emission/Eyepiece CWL/BP
DAPI	350/50	455/50
FITC	490/20	525/36
TRITC	555/25	605/52
Cy5®	645/30	*705/72
*Emission filter only		

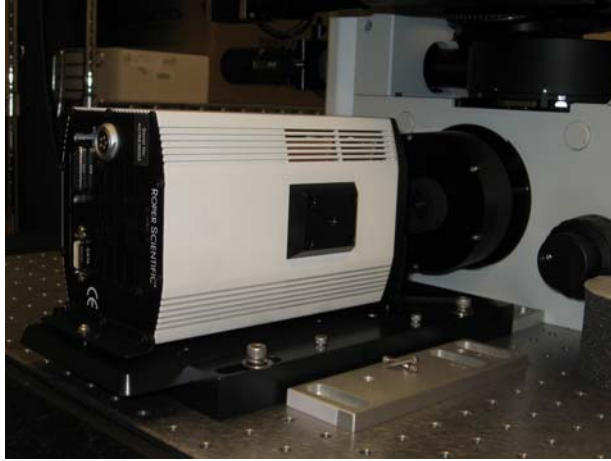
Cameras

DeltaVision Core comes standard with the CoolSNAP HQ² CCD (charge coupled device) camera, referred to as the High-speed Camera, and also supports an upgrade option of the Cascade II EM CCD camera. For more about the Cascade II camera, see EM CCD Camera on Page 107.

personalDV comes standard with the CoolSNAP ES² Camera.

High-Speed Camera

The High-Speed CCD camera (CoolSNAP HQ²) collects digital image data from the microscope. This camera is designed to collect image data at a high frame rate. At the fastest speed, the camera can collect 30 frames per second of a 64 × 64 pixel image. Increased acquisition speed is useful when collecting images of live cells that deteriorate over time.



The High-speed Camera

The High-speed Camera is air-cooled. The cooling apparatus is incorporated into the camera assembly; therefore, when the High-speed Camera is turned on, the cooler is also operating. When using cooled cameras in humid conditions, it is possible for condensation to form on the CCD camera window. If this happens, a mottled pattern is superimposed on the images. You will need to lower the ambient humidity level to avoid condensation.

Notes

#1 The High-speed Camera controller is built into the camera head. The High-Speed Camera has no user-serviceable parts inside.

#2 If no images are acquired over a 4 hour period, the High-speed Camera automatically shuts down.



CAUTION: Do not disconnect cables to the High-speed Camera when the power is on. Be sure to leave a 1 in (2.5 cm) minimum space around the cooling fan. Use only the PCI cards, cable, and power supplies that are designated for this system.

High-speed Camera Power Supply

The High-speed Camera power supply provides electric power to the camera. It is housed on the bottom shelf of the cabinet.

ES² Camera

The CoolSNAP ES² CCD Camera is basically the same as the CoolSNAP HQ² Camera, except the ES² is not as deeply cooled. This makes it more affordable, but allows for slightly higher noise. However, the noise difference is virtually unnoticeable unless you are using long exposure times (~8s).

Light Sources

DeltaVision provides two light sources: a xenon arc lamp for the main light source and a white LED for transmitted light.

Xenon Lamp

Illumination for the microscope is delivered from the xenon arc lamp to the specimen through a fiber optic cable.



The light passes through the fiber optic module (FOM), where approximately 1% of the light is diverted to the Photo sensor. The remaining 99% of the light is delivered to the microscope. For instructions on how to replace the xenon bulb, see *Replacing the Xenon Bulb* on Page 136.



WARNING: Do not disconnect the arc lamp power cable when the power is on.



WARNING: The xenon arc lamp presents potentially harmful risks to the user, including the possibility of UV exposure to skin and eyes. Before operating the microscope, consult *Chapter 2: Safety* for important information regarding arc lamp operation.



Note The illumination path alignment is critical to acquiring the highest possible image resolution. See Page Path on Page 146 for a complete description of the alignment procedure.

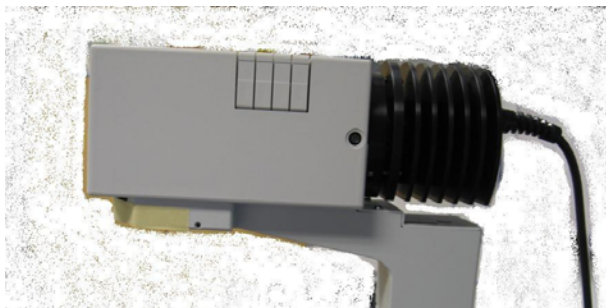
Photo sensor

The Photo sensor measures illumination intensity by sampling a small percentage of the light from the arc lamp. The photo sensor signal is recorded by the controller and then used to correct for variations in the xenon lamp intensity during an experiment.

The Corrections tool in softWoRx normalizes each image based on its photo sensor value. These corrections are automatically applied during deconvolution. This enables quantifiable intensity comparisons between images, even if the brightness of the excitation light varies between the images.

LED Transmitted Light

An LED transmitted light source is also provided.



If at any point you need to replace the transmitted light, see the instructions in *Replacing the Transmitted Light* on Page 140.



Note Either light source (the xenon lamp or the LED transmitted light) can be used with the eyepieces or the cameras.

Desktop Components

Desktop Components include the flat panel display monitor, the keyboard, the mouse, the keypad, and a joystick. DeltaVision Core includes a vibration isolation table. *personalDV* includes a bench-top microscopy isolation platform.



Flat-Panel Display Monitor

All *DeltaVision* systems are equipped with Flat-Panel LCD monitors. These monitors offer a very high level of performance in several areas pertaining to the quality of displayed images, the most critical of which is contrast ratio.

Note For instructions that show how to adjust the monitor, see the Flat-Panel Display manual.



CAUTION: *DeltaVision* is configured to work with the monitor that is included with the system. Other monitors are not necessarily supported.

The Keypad and Joystick

Many of the functions accessible through *Resolve3D* are also available on the keypad/joystick (see *Keypad/Joystick Operation* on Page 213).



Vibration Isolation Table

Within the vibration isolation table is a breadboard surface that is supported by mechanical vibration isolators. These components provide optimal performance without an external air source. The isolators are sized for the system weight as delivered. If significant additional weight is added to the breadboard, higher capacity isolation may be required. Contact Applied Precision for more information.

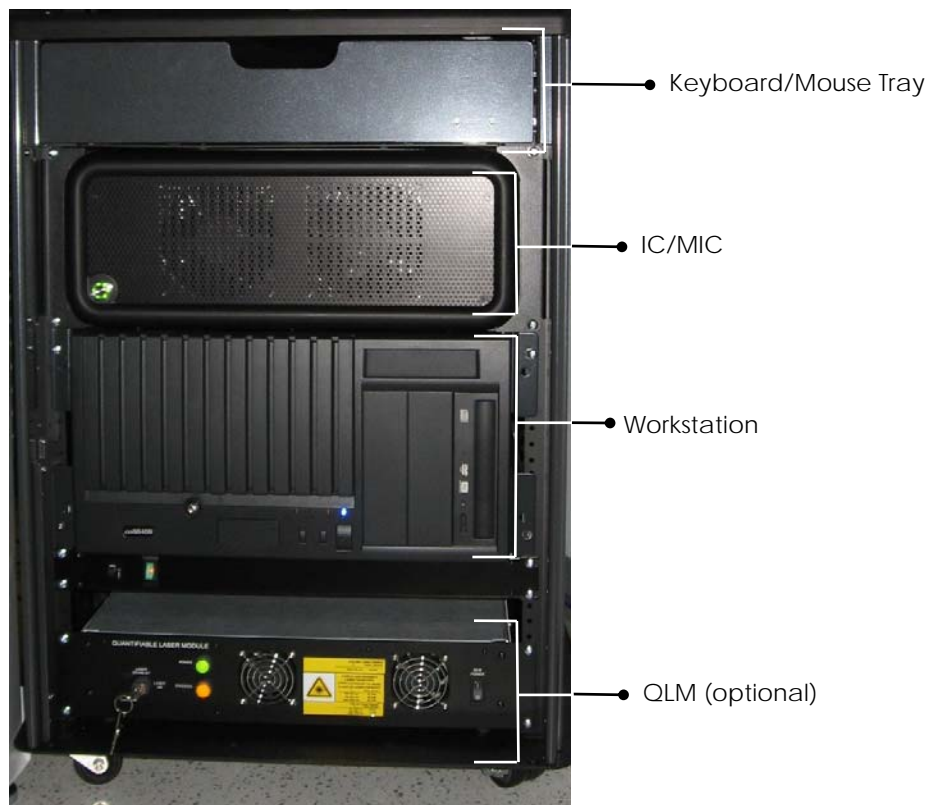
Note The vibration isolation table is not included with *personalDV*. Instead, *personalDV* includes a bench-top isolation platform to provide similar stability for the microscope. The DeltaVision vibration isolation table is available for *personalDV* as an optional upgrade.

Cabinet Components

Note The component cabinet is included with *DeltaVision Core* only. The cabinet is not available for *personalDV*.

The cabinet contains all of the electronic control equipment, the keyboard, and the Flat Panel Display. The front two wheels should remain locked and the cabinet left in place. There are many cables connecting the microscope that can be damaged if they are pulled. The standard cabinet components are shown below.

Cabinet Components



 **Note** Configuration of cabinet components may vary slightly.

Instrument Controller / Microscope Interface Chassis (IC/MIC)

The Instrument Controller (IC) is the portion of this computer that interfaces with all of the microscope hardware (including the microscope stage motors, filter wheel motors, and cameras). It coordinates all activities related to positioning the stage and collecting images. Data from the camera feeds through the controller to the workstation. The controller also receives instructions from the workstation and issues commands to the motors through the Microscope Interface Chassis.

The Microscope Interface Chassis (MIC) side of the computer provides power and control for the filter motors, stage drive, and shutters. It also contains the Photo sensor, which is connected to the microscope through a fiber optic cable.

Notes

#1 The Instrument Controller / Microscope Interface Chassis has no user-serviceable parts inside.

#2 Occasionally, you may need to replace fuses on the back panel of the chassis. Consult Chapter 9 for a description of this procedure. Unless there is an obvious reason why the fuse blew, you should contact Applied Precision when you need to replace fuses.

Workstation

The workstation hosts the *softWoRx* application that is the primary interface used to control the system. The workstation can also provide a server for data storage when used with the optional Data Management Software (DMS).

 **Note** The workstation has no user-serviceable parts inside.

DVD-R Recording

The DVD-R/CD Drive supports DVD-R format recording (DVD + R and CD-ROM are not supported.)

Other Standard Components

Other standard components include the Repeatability Slide Holder, the Slide Holder Adapter, the Fiber Optic Module, the keypad, and the joystick.

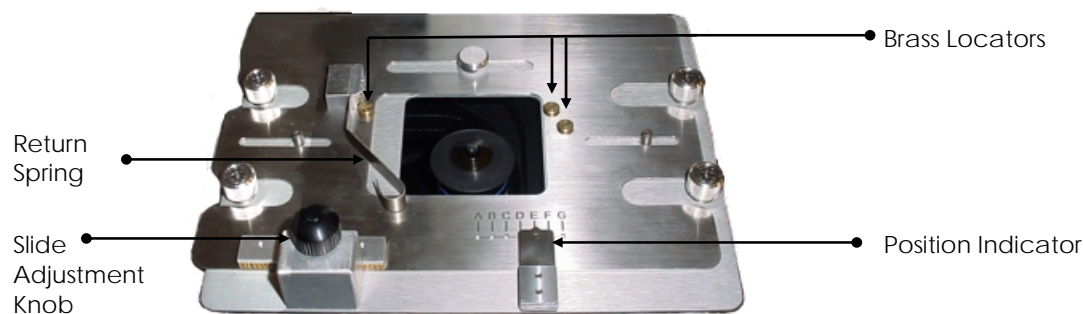
The Repeatable Slide Holder

The Repeatable Slide Holder holds the slide on the stage. It also allows you to move the slide across the stage and to mark the slide position when you remove the slide.

The ability to move the slide across the stage allows you to view the full slide on the 1" x 1" stage area. With the slide held against the three brass locators by the Return Spring, you can use the Slide Adjustment Knob to move the slide laterally.

Marking the position of your slide is useful when you are conducting a point visiting experiment and you need to remove the slide before you are finished. You can use the Position Indicator (the letter scale at the bottom of the slide holder) to record the position of your slide. When you resume your experiment, you can place the slide in the position that you recorded.

Figure 6: The Repeatable Slide Holder

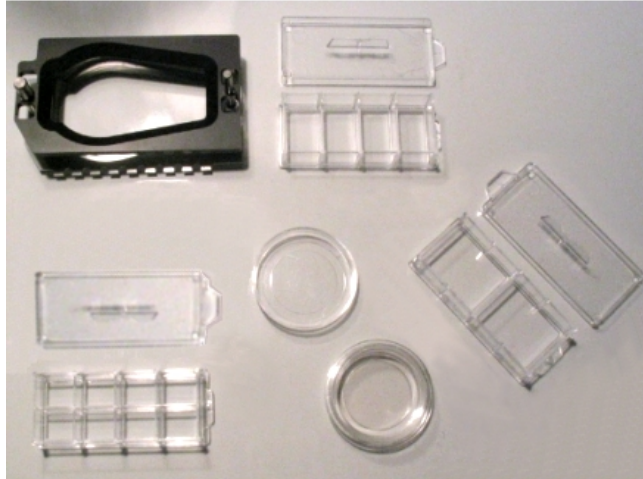


Note If you are using a Petri dish or any other format that is not similar to a 1" x 3" slide, you will need to remove the Repeatable Slide Holder or use the Slide Holder Adapter.

For more about using the Repeatable Slide Holder, see *Finding a Specimen and Recording its Position* on Page 75.

Slide Holder Adapter

The Slide Holder Adapter holds a chambered coverglass. It is mounted on the Repeatable Slide Holder. NUNC Lab-Tek™ II chambered coverglass with one, two, four, or eight wells are available. The Slide Holder Adapter also supports Petri dishes from 25 – 40 mm in diameter.



Calibration Kit

The Calibration Kit is provided for calibration operations on the DeltaVision system. The kit includes the following six slides:

- Three plastic fluorescent calibration slides – for flat-field calibration:
 - Blue plastic (EX 408nm, EM 440 nm) – Good for DAPI, Hoechst, etc.
 - Orange plastic ((EX 488nm, EM 519nm) – Good for FITC, GFP, TRITC, CY-3[®], etc.
 - Red plastic (EX 590nm, EM 650nm) – Good for CY-5[®], etc.
- Silicon mirror slide – for light path alignment and troubleshooting
- Silicon grid slide – for pixel size measurement
- 100nm Rhodamine[®] bead slide – for PSF measurement

The Fiber Optic Module

Use the Fiber Optic Module to align the light path from the fiber optic cable to the Fluorescent Illuminator. This module allows you to adjust the tilt, horizontal, and vertical orientation of the light path.



Fiber Optic Module

The Tool Kit

A tool kit is provided that includes tools for maintaining the system. This kit includes:

- Ties and belts for suspending cables, screws, and other fasteners
- Cleaning material (Q-tips and lens paper)
- A hex wrench set (5/64" – 3/16")
- A set of metric L keys (1mm –5mm)
- A #1 Phillips screwdriver
- A 5mm T Handle hex key
- An acrylic bulls eye level
- A micrometer nut wrench
- An Applied Precision Hotline sticker

Software

DeltaVision Core includes *softWoRx* for Linux (1 copy), *softWoRx Suite* for Windows (1 copy), *DMS Server* (1 copy, 3-seat), and *softWoRx DMS* (2 copies).

personalDV includes *softWoRx* for Linux (1 copy) and *softWoRx DMS* (1 copy).

Note Either system can be ordered with a number of different software configurations. Talk with your Applied Precision representative to determine the best configuration for your applications.

softWoRx

softWoRx is the Linux software application that runs the acquisition workstation. The software allows you to perform the following tasks:

- Acquire image data
- Set up and run experiments
- Deconvolve data
- Measure point spread functions
- Calculate optical transform functions
- Process 2-D and 3-D images
- Perform quantitative analysis
- Archive data
- Configure task chains
- Manage user accounts

***softWoRx* Suite for Windows**

softWoRx Suite is a Windows-based ensemble of software developed by Applied Precision in collaboration with Bitplane AG. It provides sophisticated multi-dimensional data visualization, analysis, image restoration, image correction, and image viewing management, all within an easy-to-use streamlined browser interface.

***softWoRx* DMS**

softWoRx DMS (Data Management Solution) provides a functional infrastructure for the storage of biological images and their associated metadata in a centralized database. Like *softWoRx* Suite, *softWoRx* DMS includes the Browser and the Explorer programs, but unlike *softWoRx* Suite does not have the ability to perform any type of image processing. The DMS product actually has two distinctive parts. One part is the DMS Server and the other part is represented by the DMS clients.

The DMS Server represents different configurations of what the centralized database looks like. The DMS clients are the different programs and methodologies of accessing the image data within the centralized database (ie. *softWoRx*, *softWoRx* Suite, and *softWoRx* DMS).

Optional Components

You can purchase several optional *DeltaVision* components. Some options are available for both *DeltaVision Core* and *personalDV*, but many are available only for *DeltaVision Core* (as noted below):

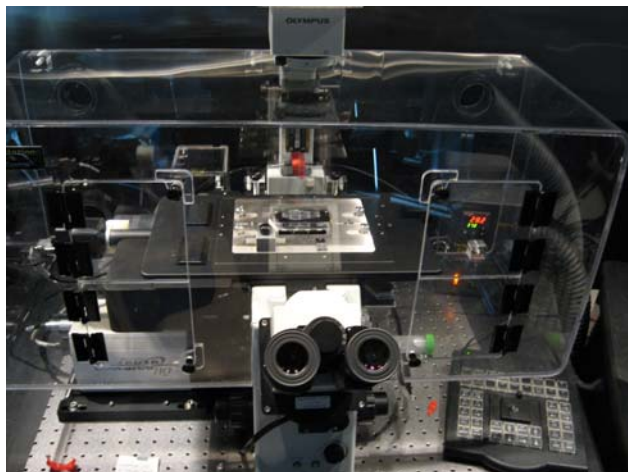
- The Environmental Chamber provides a controlled temperature environment for live cell imaging. The chamber also supports CO₂ injection, which allows you to control humidity and pH by maintaining a CO₂ flow over the sample.
- Additional Filter Modules can be loaded with custom filters or with the Live Cell filter set to provide greater flexibility for your lab.
- Quantifiable Laser Module components (available for *DeltaVision Core* only) allow you to use lasers to perform FRAP and other photokinetic experiments.
- The TIRF (Total Internal Reflection Fluorescence) module (available for *DeltaVision Core* only) includes the Olympus TIRF module and special adapters that allow you to install it on DeltaVision.
- The EM CCD Camera (available for *DeltaVision Core* only) is an optional camera that provides high signal-to-noise ratios for Low-light Fluorescence, TIRF, or single molecule fluorescence.
- Analysis Workstations include the *softWoRx* Linux workstation and the *softWoRx* Suite Windows workstation.
- Software includes two optional modules for either the *DeltaVision Core* or *personalDV*: the *softWoRx* Explorer and the *softWoRx* Suite advanced option. *softWoRx* Suite for Windows is also available as an option for *personalDV*.
- A selection of Differential Interference Contrast (DIC) components are supported by *DeltaVision Core* and *personalDV*.
- The optional Multiplexed Wavelength module for DeltaVision includes a second shuttered xenon light source and provides nearly simultaneous two-channel imaging.
- The following optional objectives are tested by Applied Precision, LLC to ensure that they meet our rigid quality standards.

Optional Objectives
U-APO 40X Oil, 0.65-1.35NA, 0.10mm WD
U-PLAN S-APO 100X Oil, 1.4NA, 0.12 WD
U-PLAN APO 60X W/PSF Water, 1.20 NA, 0.25mm WD

The following subsections describe each of the optional *DeltaVision* components.

The Environmental Chamber

The Environmental Chamber (also called the Weather Station) includes a temperature controller, a CO₂ humidifier, and a CO₂ chamber enclosed in a Plexiglas covering.



Note On some systems, the heater is on the other side of the Environmental Chamber.

For instructions that show how to install, remove, and operate the environmental chamber, see the Weather Station User's Manual (included with your system).

Additional Filter Modules

You can purchase additional *DeltaVision* Filter modules and use them to quickly change the filter sets to meet different imaging requirements.

Each filter module holds up to six filters. You can purchase a Live Cell Filter Module that is preloaded with four filter sets that are commonly used for live cell imaging. You can also purchase empty filter modules and insert customized filter sets into those modules.

The Live Cell Filter Module

The Live Cell Filter Module includes the following filters:

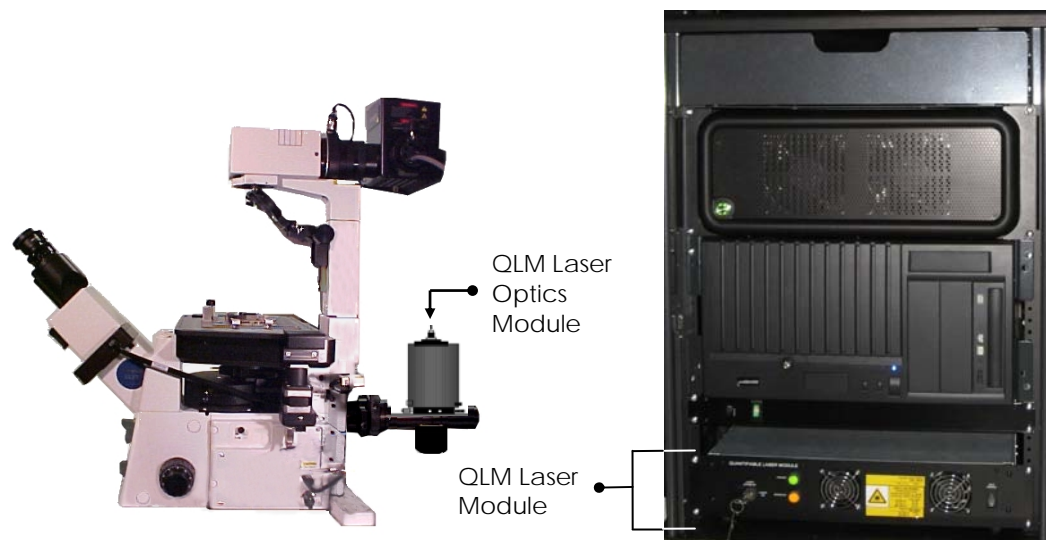
Filter Name	Excitation CWL/BP	Emission/Eyepiece CWL/BP
CFP	430/24	470/24
YFP	510/20	535/30
mCherry	572/35	632/60
EGFP	470/40	525/50

Quantifiable Laser Module Components

The Quantifiable Laser Module (QLM) adds a laser beam into the back aperture of the microscope objective to provide a focused illumination spot in the center of the optical field. The lasers are mounted in a closed system in the cabinet. Light is directed from the lasers to the light path through the Laser Optic module that mounts on the back of the Fiber Optic module.

Note The QLM is *not* an available option for *personalDV*.

If your system has the QLM hardware module, you can use *softWoRx* to analyze Photokinetic (photo-bleaching and photo-activation) experiments.

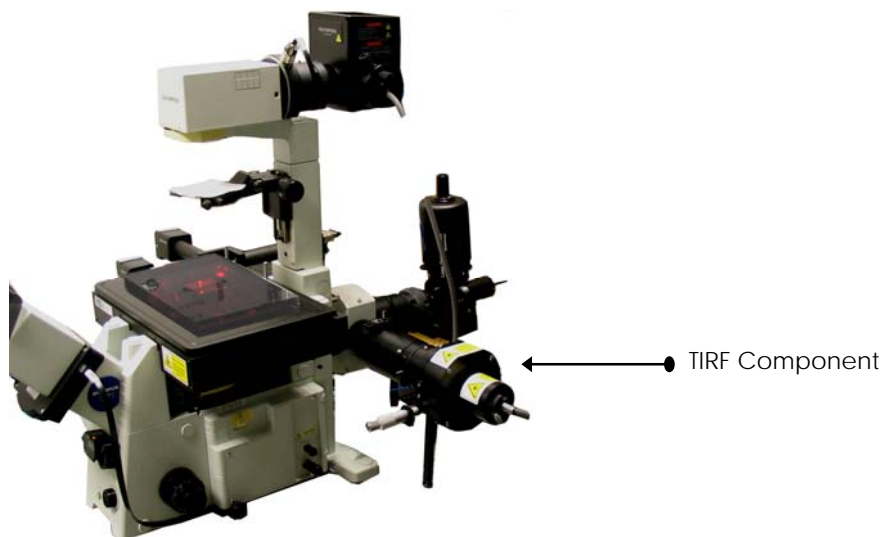



The QLM module provides software to control the lasers and to analyze the data obtained from these experiments. For more about QLM, see the *DeltaVision QLM Getting Started Guide*.

Total Internal Reflection Fluorescence (TIRF) Module

The TIRF module includes the Olympus TIRF component and special adapters required to install it on a *DeltaVision* system. (TIRF requires a system with a QLM hardware module.)

 **Note** TIRF is *not* an available option for *personalDV*.



 **Note** TIRF is an optical sectioning technique that limits fluorescence imaging to a thin area at the surface of a specimen, typically 100 nm or less, resulting in an enhanced signal-to-noise ratio and higher imaging contrast.

EM CCD Camera

The EM CCD Camera is an optional component.



 **Note** The EM CCD Camera is *not* an available option for *personalDV*.

The addition of the Cascade II with Electron Multiplying CCD (EM-CCD) technology further improves the signal-to-noise-ratio (SNR) of the *DeltaVision* system.

Unlike conventional CCD cameras, an Electron Multiplying CCD (EMCCD) is not limited by the readout noise of the output amplifier, even when operated at high readout speeds. A solid state Electron Multiplying (EM) register allows weak signals to be multiplied before any readout noise is added by the output amplifier.

The EM CCD camera can be operated either as a high-performance EMCCD camera for unparalleled low-light-level sensitivity or as a traditional, non-electron-multiplying CCD camera.

Applications for the EM CCD camera include:

- Low-light Fluorescence
- Total Internal Reflection Fluorescence Microscopy (TIRF)
- Single-molecule Fluorescence

Analysis Workstations

You can purchase two types of Analysis workstations: the *softWoRx* Linux workstation and the *softWoRx* Suite Windows workstation.

The *softWoRx* Linux workstation includes all of the *softWoRx* Analysis modules, including 3D Visualization tools, Colocalization, FRET Analysis, FRAP Analysis, Intensity and Distance Measurement, and Modeling.

The *softWoRx* Suite Windows workstation includes the *softWoRx* Browser, *softWoRx* Explorer, Modeling and 3D Visualization (powered by Bitplane), and Deconvolution tools.

Multiplexed Wavelength Module

The optional Multiplexed Wavelength module for the *DeltaVision* systems allows you to perform nearly simultaneous two-channel imaging without the drawbacks associated with true simultaneous two-channel imaging. This option uses two shuttered illumination sources and a dual-band emission filter to eliminate filter wheel movement between channels, and therefore greatly reduce the time required for the *DeltaVision* system to acquire a set of two-channel images. The combined light path ensures no registration artifacts are introduced and independent excitation of probes helps to ensure minimal crosstalk.

Software

softWoRx Explorer

softWoRx[®] Explorer is a cross-platform image viewer that is available for the following operating systems:

For 32 bit systems

- Apple Mac OSX 10.2, 10.3, and 10.4
- Microsoft Windows 2000 Professional, XP Home, or XP Professional
- Red Hat Enterprise Linux 2.1 - 4.0 with Gnome or KDE interface
- SuSe 8.2 - 9.2

For 64 bit systems

- Linux Red Hat Enterprise 3.0 - 4.0
- SuSe 8.2 - 9.2
- Microsoft Windows XP Professional x64 Edition

softWoRx Explorer allows you to view and explore *DeltaVision* images and images from other sources that contain spatial, temporal, and spectral ranges. In addition to displaying data in the X and Y plane, you can scroll through Z sections and time-lapse data. Individual spectrum (i.e., channels or fluorescent wavelengths) can be hidden or displayed in a variety of colors.

softWoRx Suite for Windows Option (for personalDV)

softWoRx Suite is a Windows-based ensemble of software developed by Applied Precision in collaboration with Bitplane AG. It provides sophisticated multi-dimensional data visualization, analysis, image restoration, image correction, and image viewing management, all within an easy-to-use streamlined browser interface.

softWoRx Suite Advanced Option

The Advanced Option for *softWoRx Suite* includes two sophisticated analysis features: *4-D Particle Tracking* and *ImarisColoc*. *softWoRx Suite Advanced Option* is offered by Applied Precision in collaboration with BitPlane, AG.

4-D Particle Tracking allows users to observe temporal changes of objects. This tracking module offers a choice of methods for both detection and tracking and allows analysis and measurement of various object properties.

The Colocalization feature enables users to easily isolate, visualize, and quantify regional overlap in 3D and 4D images. Results can be presented in two ways—as a new 3D or 4D channel or as a statistical report.

***softWoRx* Data Management Solution (DMS) Support**

softWoRx DMS provides a functional infrastructure for the storage of biological images and their associated metadata in a centralized database. *softWoRx* DMS is a standard component included with *DeltaVision Core*, but for *personalDV* this software is optional.

Consumable Parts

Common to 100-120 V and 220-240 V Systems

Component	Fuse for Component	APLLC Part Number
IC/MIC	6.3A 250V UL High Break Capacity	19-170045-000
Microscope	T5AH	Used in some older DeltaVision systems

Bulbs

Component	APLLC Part Number
250W xenon Arc Lamp Bulb	34-100390-000
LED Transmitted Light (must replace entire assembly)	52-851243-000

British Power Cord

Component	Fuse for Component	APLLC Part Number
Power cord with Bussman TDC180-10A built into plug. (Must meet British Standard BS1362.)	10A	19-210046-000

8

Changing Cameras and Filters

This chapter provides the following instructions for cameras and filters:

- Changing Cameras
- Using Live Cell or Custom Filter Wheel Modules
- Changing Filter Wheel Modules
- Calibrating the Filter Wheels

Changing Cameras

DeltaVision Core includes a High-speed Camera as a standard component. If you have the optional EM CCD camera, you can change cameras to better meet your imaging needs.

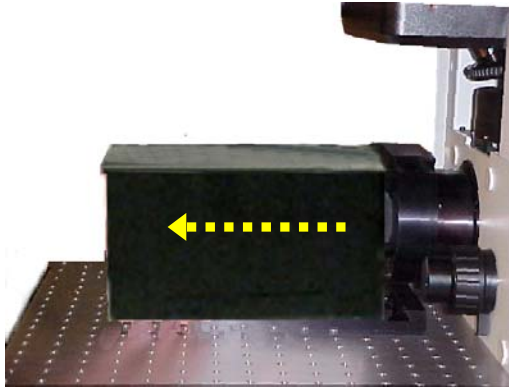
These instructions show how to change the camera and how to select the new camera in Resolve3D.



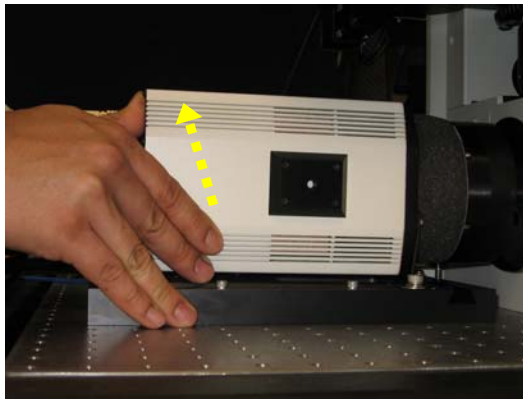
CAUTION: Contamination from fingerprints or dust on the camera window or inside the Emission filter wheel will degrade image quality.

To change the camera:

1. Remove the camera cover by lifting it up and sliding it away from the microscope.



2. Remove the camera by pushing in and then pulling up on the camera end. You do not need to remove cables from the camera.



3. Remove the other camera from the camera tray and place the camera that you just removed on the camera tray.



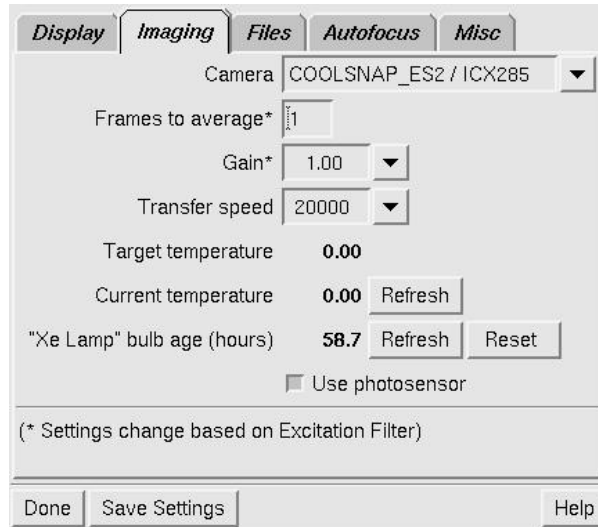
CAUTION: Always place the camera in the camera tray when it is not secured to the system. Dropping the camera will cause severe damage.

4. Install the new camera by sliding it into place and then pushing in and pressing down on the end of the camera.
5. Replace the camera cover.

To select the camera:

1. In the Resolve3D window, click **Settings** to open the Resolve3D Settings dialog.

2. On the Imaging tab, click the Camera list and select the camera that is currently installed.



Notes

#1 The EM CCD camera is listed twice in the Camera list:

CASCADE2_512 Conv./... sets the camera in Conventional mode.

Cascade2_512 EMCCD/... sets the camera in electron-multiplication mode.

#2 When the camera is changed, the binning number is reset to 1.

#3 If the camera cables loosen or are accidentally disconnected, you may need to restart the IC software. To reseal the cables, consult the camera documentation.

Using Live Cell or Custom Filter Wheel Modules

A filter wheel module includes an Excitation filter wheel, an Emission filter wheel, and an Eyepiece filter wheel.

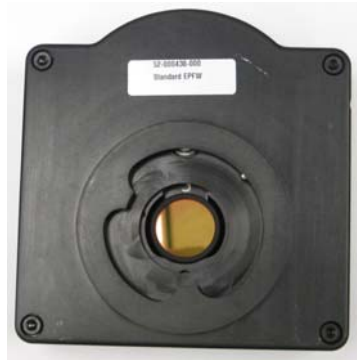
DeltaVision Filter Wheel Module



Excitation filter wheel



Emission filter wheel



Eyepiece filter wheel

A filter wheel module includes the Excitation, Emission, and Eyepiece filter wheels and can hold up to six filter sets.

If your system has an alternate filter wheel module, you can swap modules to meet your imaging needs.

You can purchase a module of Live Cell filter wheels that includes factory-installed filters or you can purchase a module of empty filter wheels and customize it with your own filters.

Changing Filter Wheel Modules

Changing the filter wheel modules for your system includes changing the Emission, Excitation, and Eyepiece filter wheels.

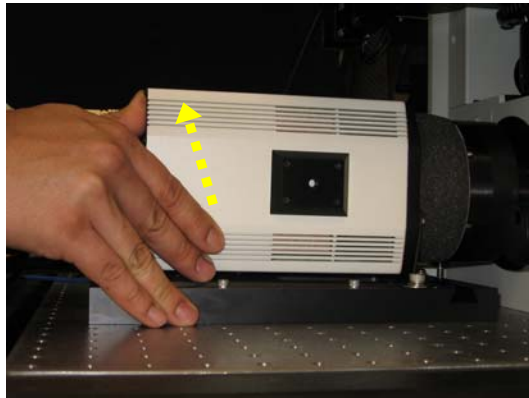
If you purchased a filter wheel module as a separate component after you purchased your system, you must configure the new module the first time that you use it (see the instructions that are included with your filter wheel module).



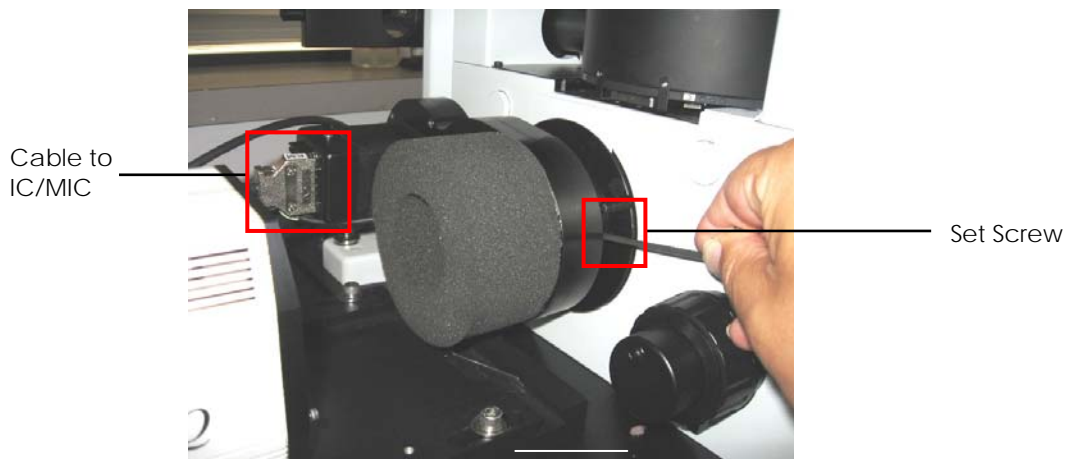
WARNING: Before you start, make sure that the xenon lamp and the IC/MIC are turned OFF.

To change the Emission filter wheel:

1. Turn off the IC/MIC.
2. Remove the camera cover. Then remove the camera by pushing in and pulling up on the camera end as shown below.

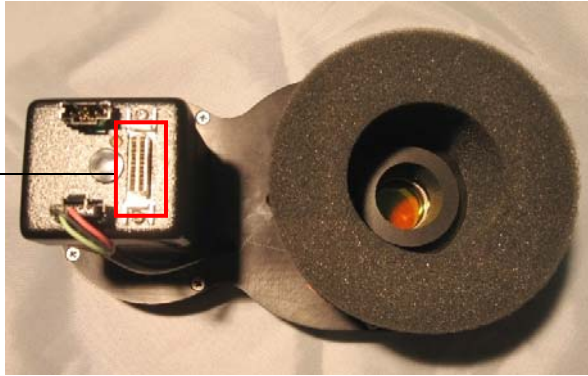


3. On the Emission filter wheel motor, disconnect the cable that connects the motor to the IC/MIC. Use the Olympus 3 mm hex key to loosen the set screw that holds the Emission filter wheel to the Shutter Assembly.

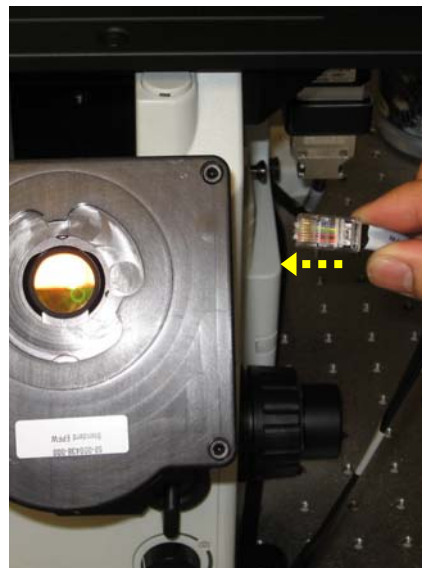
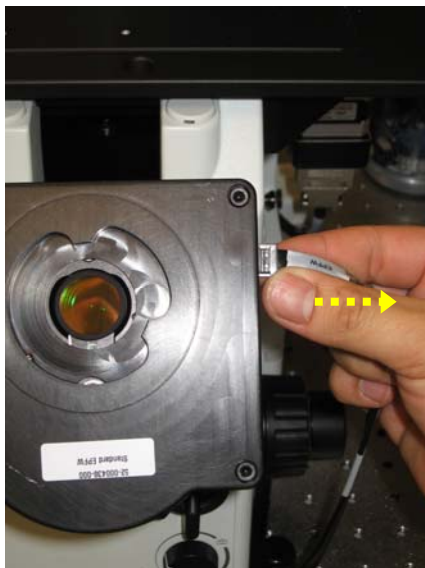


4. Remove the Emission filter wheel.
5. Connect the cable from the IC/MIC to the new Emission filter wheel.

IC/MIC Cable
Connector



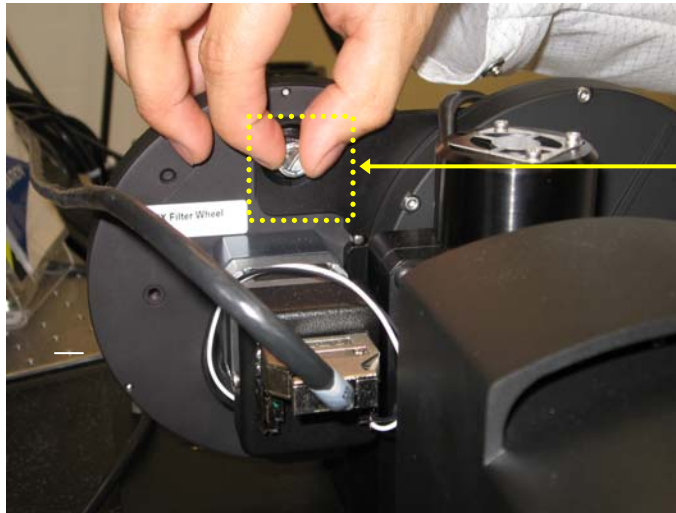
6. Install the alternate Emission filter wheel. Tighten the Emission filter wheel set screw.
7. Reinstall the camera by sliding it into place and then pushing in and pressing down on the camera.
8. When you are finished changing filter wheels, restart the IC/MIC.
9. There are two ways to let the system know you have a new filter set:
 - From the Resolve3D main menu, select **Settings**, change to the Misc tab, and select the current filter set.
 - Unplug and then re-plug in the Eyepiece filter wheel position sensor cable (see below). The system will automatically recognize which filter set the Eyepiece filter wheel belongs to and update accordingly.



Unplug and re-plug in the Eyepiece filter wheel

To change the Excitation Filter Wheel:

1. Ensure that the IC/MIC is turned off.
2. Turn off the xenon arc lamp and allow it to cool.
3. Loosen the two silver thumb screws at the top and bottom of the filter wheel housing.

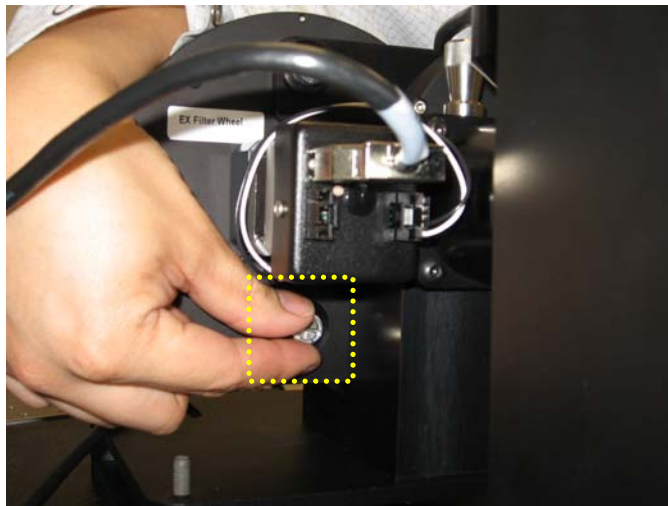


Thumb screws should loosen without the use of tools, but can also be removed with a flat-blade screwdriver if necessary.

Loosening top thumb screw from filter wheel housing

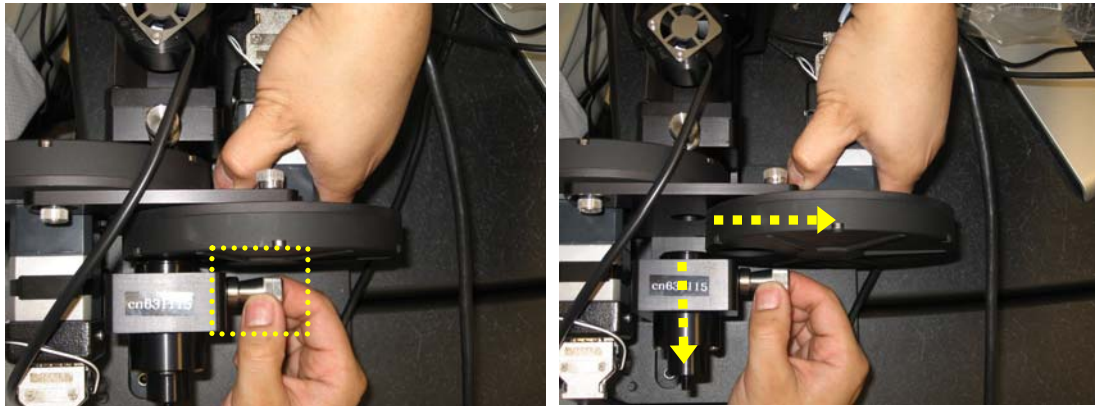


CAUTION: Do not bend the fiber optic cable into a coil with a diameter less than 24".



Loosening bottom thumb screw from filter wheel housing

4. Loosen the Focusing Lens housing thumb screw to disengage it from the beveled support on the Excitation filter wheel housing and gently slide the filter wheel housing outward from its mounted position.



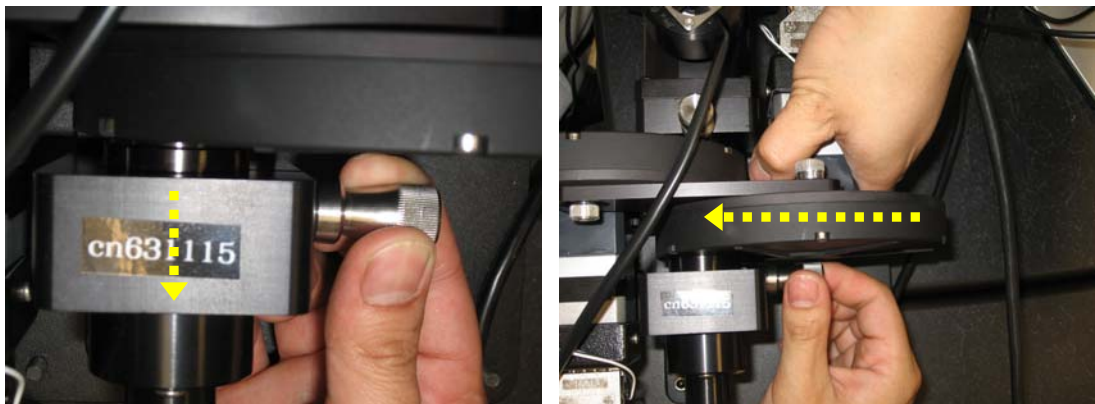
Removing the Excitation Filter Wheel housing from its mounted position

5. Disconnect the cable to the IC/MIC from the Excitation filter wheel and connect it to the alternate Excitation filter wheel.



Disconnecting the IC/MIC cable from the Excitation Filter Wheel

6. Slide the alternate Excitation filter wheel housing back into its mounting position by pulling back on the Focusing Lens assembly as shown.



Pulling back the Focusing Lens housing and re-engaging the filter wheel

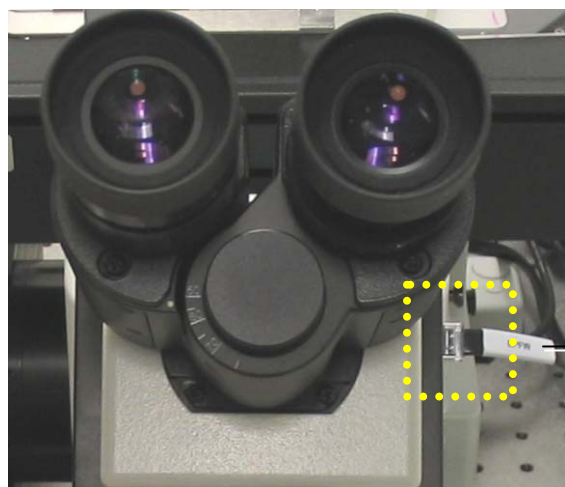
7. Re-engage the Focusing Lens housing into the beveled support on the new Excitation filter wheel housing and align and tighten the two thumb screws at the top and bottom of the Excitation filter wheel housing.
8. Tighten the thumb screw on the Focusing Lens housing.
9. When you are finished changing filter wheels, restart the IC/MIC.
10. There are two ways to let the system know you have a new filter set:
 - From the Resolve3D main menu, select Settings, change to the Misc tab, and select the current filter set.
 - Unplug and then re-plug in the Eyepiece filter wheel position sensor cable (see Step 9 in the previous procedure). The system will automatically recognize which filter set the Eyepiece filter wheel belongs to and update accordingly.

To change the Eyepiece filter wheel:



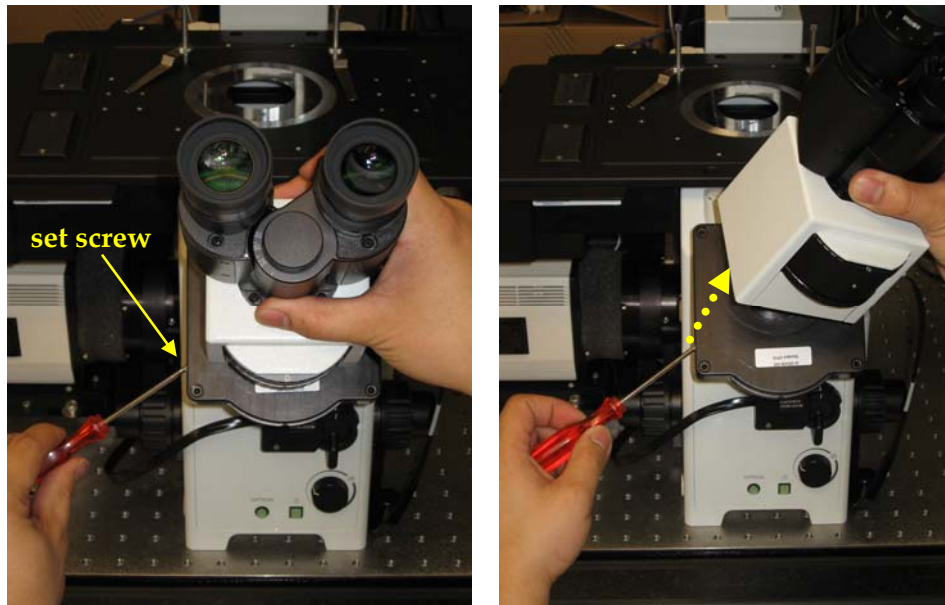
CAUTION: Do not change the Eyepiece filter wheel until you have replaced the Excitation and Emission filter wheels and connected their respective cables. Installing the Eyepiece filter wheel when these filter wheels are not connected will cause a fatal error.

1. Disconnect the Position Sensor cable that connects the filter wheel to the IC/MIC. *DeltaVision* displays a message that indicates the cable is removed. Do not close this message.



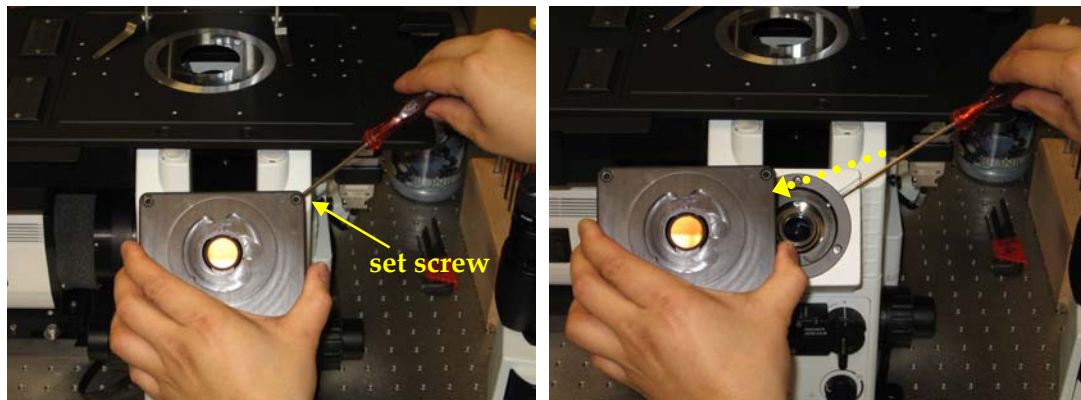
Position Sensor Cable

2. Holding the oculars in one hand, use the Olympus 3 mm hex key to loosen the set screw that holds the oculars to the Eyepiece filter wheel and set the oculars on the table.



Loosening the set screw and removing the oculars

3. Loosen the set screw that holds the Eyepiece filter wheel to the beveled mount on the stand and remove the Eyepiece filter wheel.



Removing the Eyepiece filter wheel

4. Place the new Eyepiece filter wheel on the beveled mount on the stand and tighten the set screw that holds it in place.
5. Connect the Position Sensor cable to the new Eyepiece filter wheel.
6. Place the oculars on the microscope and tighten the set screw that holds them in place.

7. If the IC/MIC is on, click **OK** on the message box that indicates the cable is removed. When the message closes, the system is ready only if the position sensor is working.
If the IC/MIC is off, you will need to manually update the filter set in the Resolve3D | **Settings** | Misc tab window.

Calibrating the Filter Wheels

Calibration initializes the filter positions and ensures that the filters are centered in the filter wheel openings.

If you notice poor light transmittance or poor image quality, one possible cause is a misaligned filter wheel. The filter wheel calibration re-establishes the zero position of the filter wheel for the Instrument Controller.

To calibrate the Neutral Density, Excitation, and Emission filter wheels, contact Applied Precision Customer Service at:

Phone: 800-862-5166

email: servicehotline@apllc.com

Hours: 8:00 AM – 5:00 PM, Pacific Time, Monday – Friday

Setting up Filter Wheels

Configuring a Live Cell Filter Wheel

If you purchased a Live Cell Filter Wheel Module as a separate component after you purchased your system, follow these instructions to configure the new filter wheel module set.

If you purchased the Live Cell Filter set with a new system, you do not need to set it up it. (New systems that include Live Cell modules are factory configured and calibrated.)



WARNING: Before you start, make sure that the xenon arc lamp is off.

To configure a new Live Cell filter wheel module set:

1. Quit the IC540 application as follows:

- a) If the Linux desktop is displayed, press Scroll Lock on the keyboard twice and then press the up arrow.
 - b) Press the Esc key twice.
 - c) When you are prompted to exit, type **Y**.
2. Double-click the **Instrument Controller** desktop folder to open it.
 3. Double-click the **Controller Configuration** icon to open the `ic540.ini` file.
 4. Create a backup file by renaming and saving the file. (Create the new name by adding an underscore and the date in the format `_MMDDYY` to the file name.)
 5. Close and reopen the original file. (Verify that you are working in the original file and not the backup that you just created.)



Caution As you edit this file, do not use the tab key; the tab key will cause errors. Use the space bar to create spaces as necessary.

6. Scroll down to the [Filter Wheel Configuration] section and enter the number of extra filter sets that your system will have after the new filters are added. Enter this number after Number of Extra EM Filter Sets, Number of Extra EX Filter Sets, and Number of Extra EP Filter Sets as shown below:

```
[Filter Wheel Configuration]
Number of ND Filter Wheels = 1
Number of EX Filter Wheels = 1
Number of EM Filter Wheels = 1
Number of EP Filter Wheels = 1
Number of Extra EM Filter Sets = 1
Number of Extra EX Filter Sets = 1
Number of Extra EP Filter Sets = 1
Active EM Filter Set ID = 1
Active EX Filter Set ID = 0
Active EP Filter Set ID = 0
```



Notes This is the number of *extra* filter sets. If you add one set to the standard set, there is one extra set.

Filter wheels are also called filter sets.

7. Verify that the file includes the following Emission filter settings for the Live Filter Set. These settings are typically below the Emission Filter Wheel section:

```
[Extra Emission Filter Set 1]
Filter Set Name = Live Cell
Number of Filters = 6
Filter Names =      BLOCK1  CFP      YFP      GFP      mCherry  POL
Filter Values 1 =   0.00   470.0   535.0   525.0    632.0    0.0
Filter Values 2 =   0.00    24.0    30.0    50.0     60.0     0.0
Filter Pos =       0.00     1.0     2.0     3.0      4.0      5.0
```

Home Position Offset = 0.0



Note You can cut and paste these settings from the .pdf file that includes these instructions to the ic540_dv.ini file.

8. Verify that the file includes the following Eyepiece filter settings for the Live Filter Set. These settings are typically below the Eyepiece Filter Wheel section:

```
[Extra Eyepiece Filter Set 1]
Filter Set Name = Live Cell
MIC ID = 1
Number of Filters = 6
Filter Names =      BLANK      POL      CFP      YFP      GFP      mCherry
Filter Values 1 =   0.00      0.00    470.0    535.0    525.0    632.0
Filter Values 2 =   0.00      0.00     24.0     30.0     50.0     60.0
Filter Pos =        0.00      1.0      2.0      3.0      4.0      5.0
Home Position Offset = 0.00
```

9. Verify that the file includes the following Excitation filter settings for the Live Filter Set. These settings are typically below the Excitation Filter Wheel section:

```
[Extra Excitation Filter Set 1]
Filter Set Name = Live Cell
Number of Filters = 6
Filter Names =      BLOCK1    BLOCK2    CFP      YFP      GFP      mCherry
Filter Values 1 =   0.00      0.0      430.0    500.0    470.0    572.0
Filter Values 2 =   0.00      0.0      24.0     20.0     40.0     35.0
Filter Pos =        0.00      5.0      6.0      7.0      8.0      9.0
Home Position Offset = 1.0
```

10. Save the ic540_dv.ini configuration file.

Setting up Custom Filter Wheel Modules

Use the following instructions to set up a customized filter module. First, install filters in an empty set of Excitation, Emission, and Eyepiece filter wheel modules. Then configure DeltaVision for the new filters.



WARNING: Before you start, make sure that the xenon arc lamp is off.

Before you Start

Set up a table that identifies the name, wavelengths, position number, and Eyepiece filter wheel label for each filter. The following example table is based on the Live Cell Filter Wheel module. Make sure that you leave the first position blank so that *DeltaVision* can identify the initial position.

Table 3: Examples of Filter Positions

Filter Name	Excitation CWL/BP*	Emission/Eyepiece CWL/BP	Filter Positions	Eyepiece Filter Wheel Label**
Blank			0	1
CFP	436/10	465/30	1	2
YFP	492/18	535/30	2	3
dsRED	580/20	630/60	3	4
EGFP	470/40	525/50	4	5
Blank			5	6

CWL is wavelength and BP is bandpass

** The label on the Eyepiece Filter Wheel is offset by one number from the filter position that you use for the `ic540_dv.ini` file. For example, the CFP filter is inserted into the slot labeled 2 on the Eyepiece Filter Wheel, but the CFP filter position in the `ic540_dv.ini` file is 1.

Use the following table to set up custom filter positions:

Table 4: Custom Filter Table

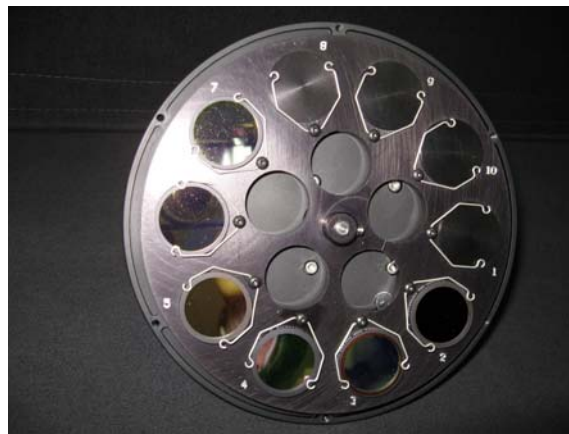
Custom Filter Table

Filter Name	Excitation CWL/BP	Emission/Eyepiece CWL/BP	Emission and Excitation Filter Position	Eyepiece Filter Position
Blank			0	1
			1	2
			2	3
			3	4
			4	5
Blank			5	6

* CWL is wavelength and BP is bandpass

To install custom excitation filters:

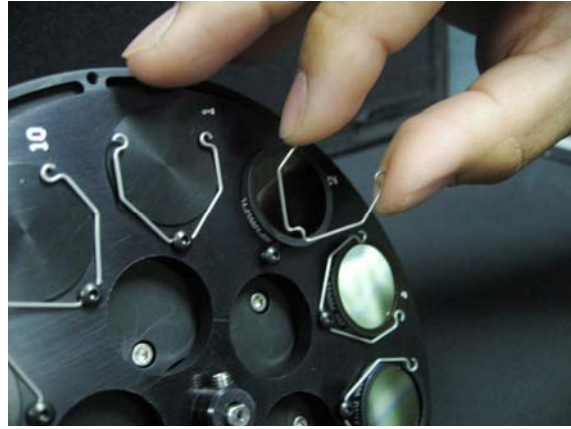
1. Use a hex key to loosen the 6 screws on the Excitation filter wheel cover and remove the cover.



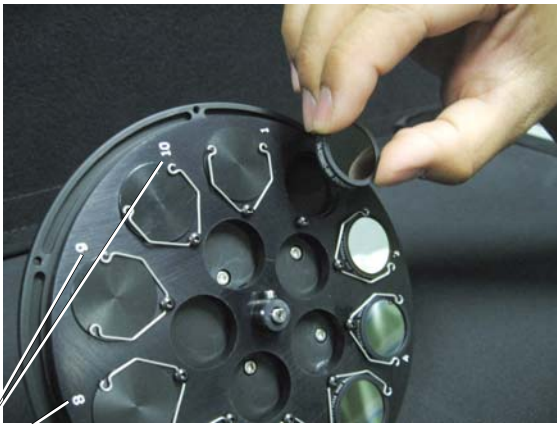


Note Avoid contact between the filters and your fingertips. If necessary, wear latex gloves for this procedure.

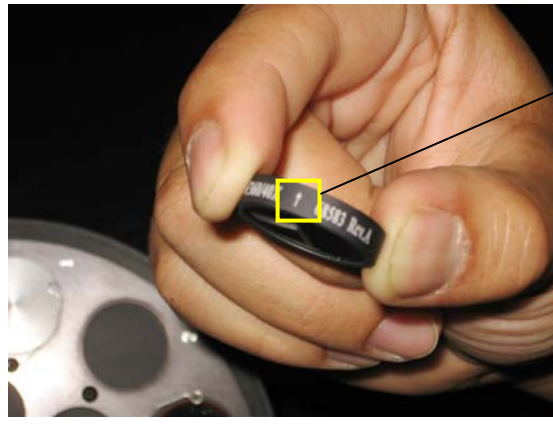
2. On the Filter Wheel, find the position number for the filter that you are installing (as listed on your Customized Filter Table). Use a hex key to loosen the screw of the spring clip for that filter, and then remove the spring clip.



3. Grip the filter by its edges and carefully remove it from the filter wheel.



Filter
position
numbers



Filter
orientation
arrow

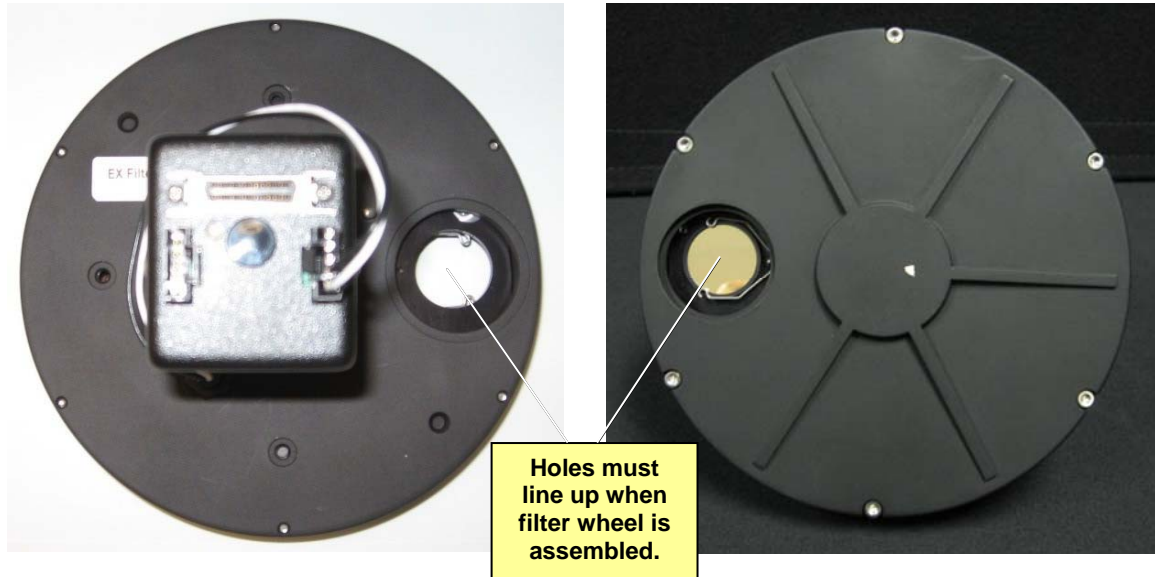
4. Slip the new filter into the empty position. The filter should be placed into the open pocket between the arms of the spring clip, with the arrow on the filter pointing up (toward the filter wheel cover).



5. Tighten the spring clip screw, making certain the spring clip arms are set properly over the filter as shown.

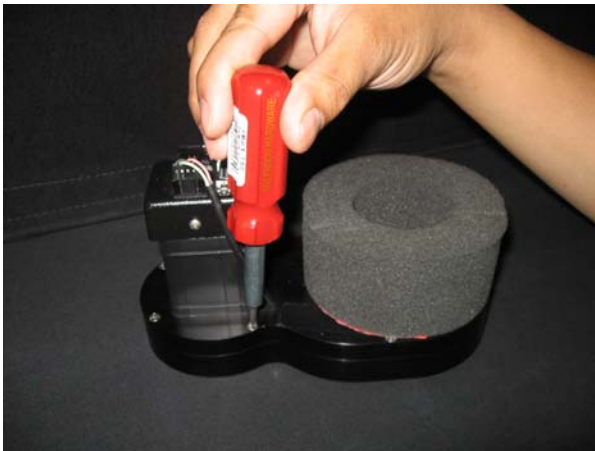


6. Repeat Steps 2 through 5 for each Excitation filter you are installing.
7. When you replace the filter wheel cover, be sure the hole in the cover lines up with the hole in the filter assembly. Then tighten the six hex screws.



To add emission filters:

1. Use a #1 Phillips screwdriver to loosen the six screws on the Emission Filter Wheel cover and remove the cover.



Note Avoid contact between the filters and your fingertips. If necessary, wear latex gloves for this procedure.

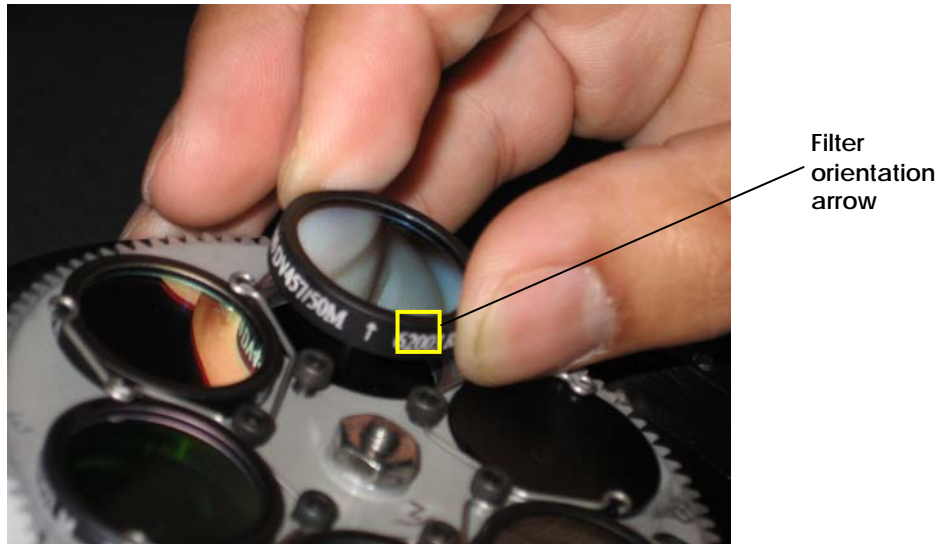
2. On the Filter Wheel, find the position number for the filter that you are installing (as listed on your Customized Filter Table). Use a hex key to loosen the screw of the spring clip for that filter.



3. Remove the spring clip and carefully remove the filter by its edges.



4. Place the new filter into the empty pocket, making certain the arrow on the edge of the filter is pointing up (toward the filter cover).



5. Replace the spring clip and tighten the hex screw. Then, adjust the arms of the spring clip so that it slightly rides up on the metal ring of the filter as shown.



6. Repeat Steps 2 through 5 for each Emission filter you are installing.
7. When finished installing filters, replace the Emission Filter Wheel cover and tighten the six Phillips screws.



To add eyepiece filters:

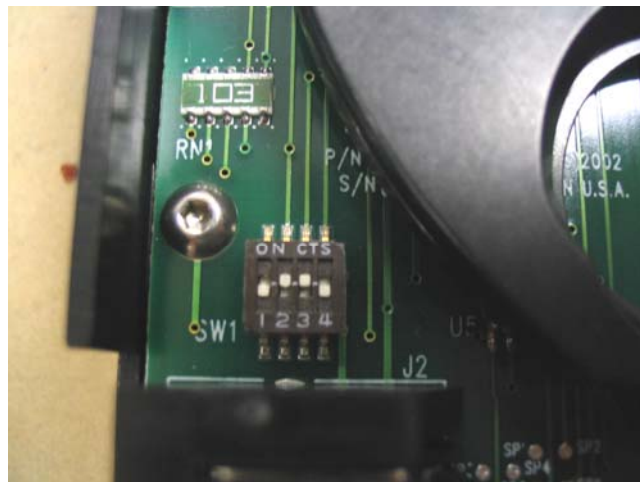
1. Locate the 3mm hex key provided with the microscope.
2. Loosen the four screws that hold the module together and remove the front cover of the module.



3. Find the filter position number for the filter that you are installing.



- 4 Drop the new filter into the empty filter holder at an angle so that the o-ring holds the filter in place. Be sure to orient the filter as follows:
 - For Chroma or excitation filters, the filter must be oriented so that the arrow points in to the direction of the light path (or down).
 - For Omega filters, the filter must be oriented so that the arrow points out from the direction of the light path (or up).
- 5 Repeat Step 4 for each filter that you are adding.
- 6 If you are installing a second customized filter wheel module, set the number 1 and 4 DIP switches on the filter wheel down and the 2 and 3 DIP switches up as shown below. (This is only required when your system has two customized filter wheel modules. If you have only one set of customized filter wheel modules, you can skip this step.)



- 7 Reassemble the Eyepiece Filter Wheel Module.

9

Maintenance

This chapter provides the following instructions for the basic maintenance of the system:

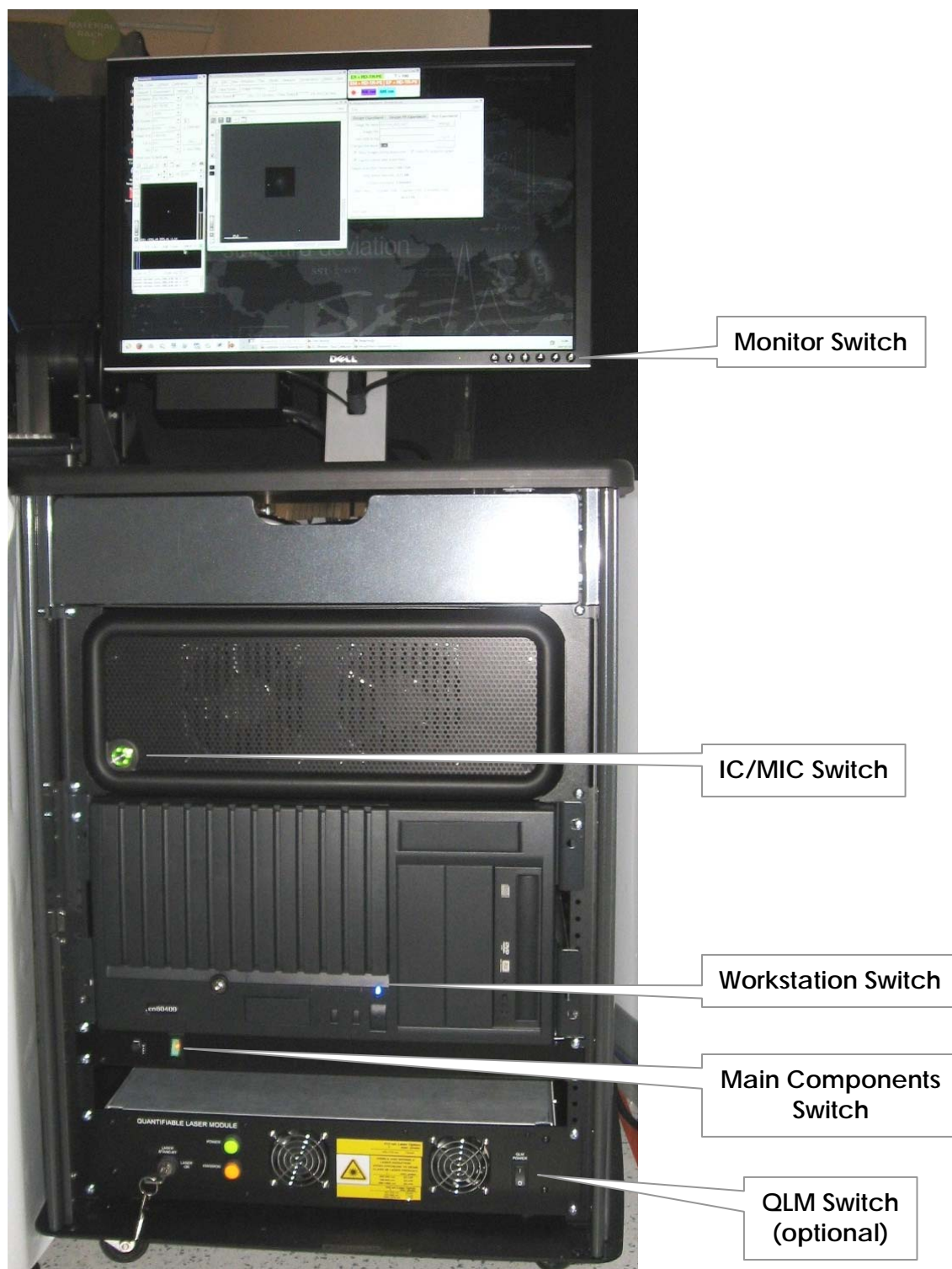
- Shutting Down and Starting the System
- Replacing the Xenon Bulb
- Replacing the Transmitted Light
- Aligning the Illumination Path
- Replacing IC/MIC Fuses
- Cleaning
- Moving the System

Shutting Down and Starting the System

Use the following instructions for shutting the system down during a power outage or other occasions that require total shutdowns.

DeltaVision Power Switches

The main *DeltaVision* power switches are shown below. (The Master Switch on the isolation transformer that plugs into the wall is not shown.)



Guidelines for Using Switches

Main Components

Use this switch to turn power on and off for the *DeltaVision* Workstation, Instrument Controller and Microscope Interface Chassis (IC/MIC), the Fast Camera Power Supply, and the *DeltaVision* Microscope.



Workstation, IC/MIC, and Monitor

Leave these switches on except on rare occasions (such as power outages) when you need to shut down the entire system.

Shutting Down the System

In some situations, such as power outages, you will need to shut down the entire *DeltaVision* system.

To shut down the *DeltaVision* system:

1. Save all data on the workstation.
2. Turn off the xenon light source using the bulb  icon on the Resolve3D main menu. Clicking the icon will switch it to the off  state.
3. On the *softWoRx* menu bar, choose **File | Exit**. Then exit all other workstation applications.
4. From the main menu button, choose **Logout** and then **Shut Down**. Wait until the monitor displays *Power Down*.
5. Press the power button on the IC/MIC once to shutdown.
6. Turn off the monitor.
7. Turn off the power strip bar switch.

 **Note** For *personalDV*, there is no power strip bar. For *personalDV*, skip this step.

8. Clean the objective (see *Turning DeltaVision Off* on Page 28).
9. Lower the objective.

Starting the System

Use the following instructions to start the system after a total shut down.

To start the *DeltaVision* System:

1. Turn on the power strip bar.

Note For *personalDV*, there is no power strip bar. Begin the power-up process with Step 2.

2. Turn on the IC/MIC.
3. Turn on the workstation.
4. Turn on the monitor.
5. Follow the instructions for turning on *DeltaVision* on Page 17.

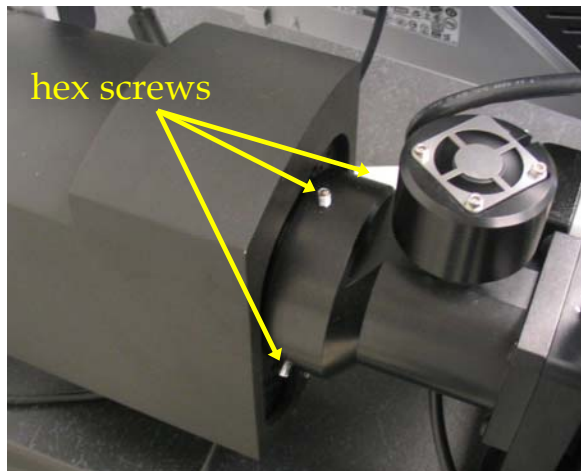
Replacing the Xenon Bulb



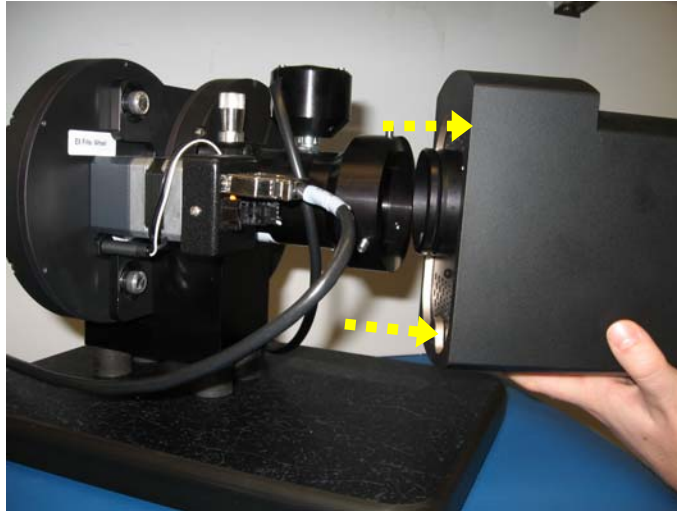
WARNING: Ensure the xenon lamp is off and has had plenty of time to cool before starting this procedure.

Follow these steps to replace the xenon bulb on *DeltaVision*:

1. If the system is on, exit Resolve3D and ensure that the IC/MIC is off prior to proceeding. The fan on the lamp housing must be off before you begin this procedure.
2. Loosen the three hex screws in the flange of the xenon lamp housing.



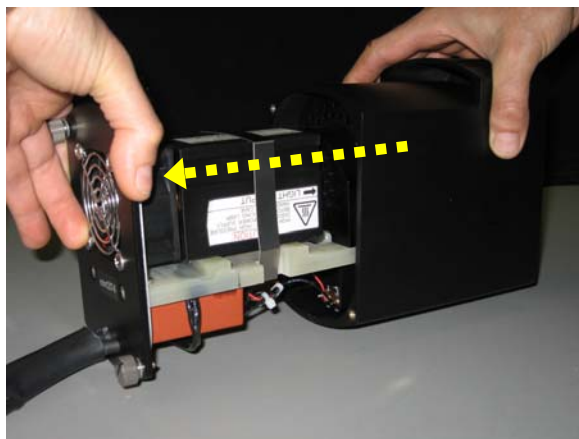
3. Gently slide the lamp housing away from the flange to remove it from the *DeltaVision* excitation module.



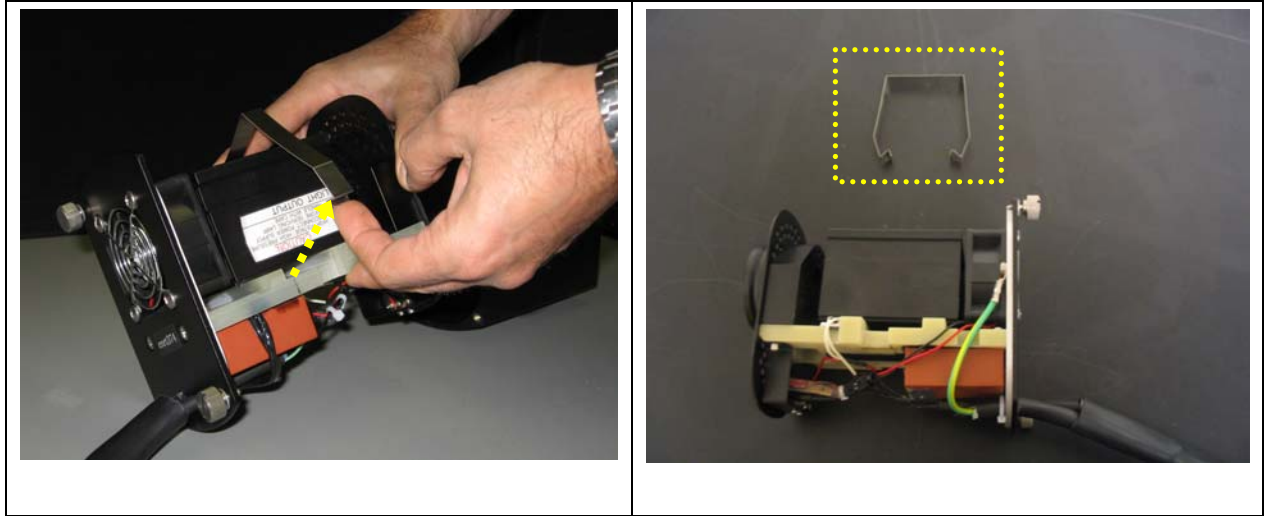
4. Loosen the two thumb screws on the opposite end of the xenon lamp housing.



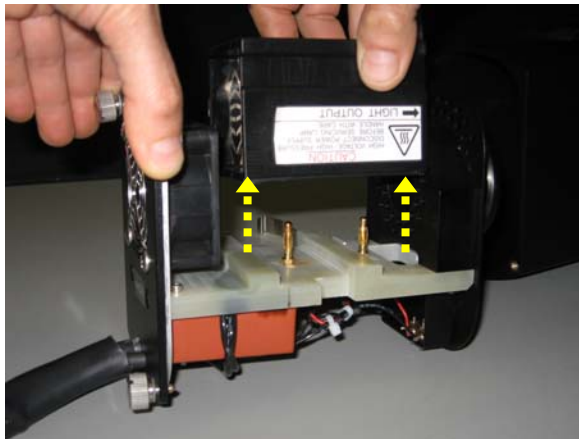
5. Gently slide the internal lamp mechanism from the lamp housing.



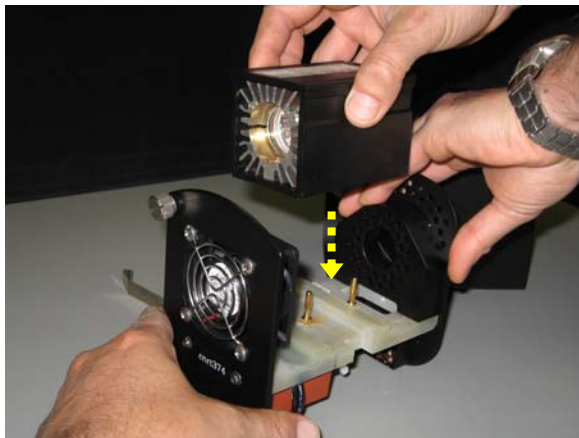
6. Remove the center clip from the internal lamp mechanism as shown.



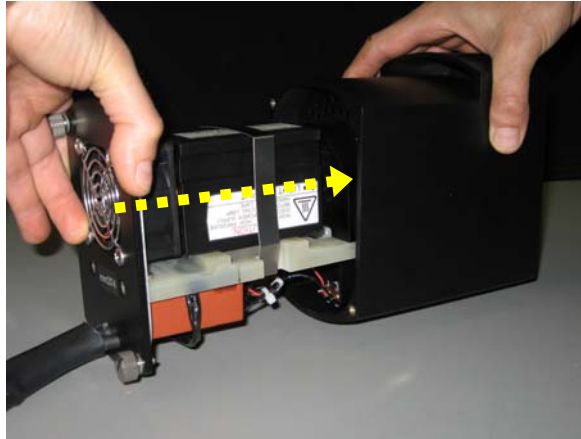
7. Lift the bulb assembly (small black box) from the two supporting pins in the lamp mechanism.



8. Replace the xenon bulb assembly with a new one (Part #34-100390-000).



9. Replace the clip around the internal lamp assembly and gently slide the internal lamp mechanism into place within the lamp housing.



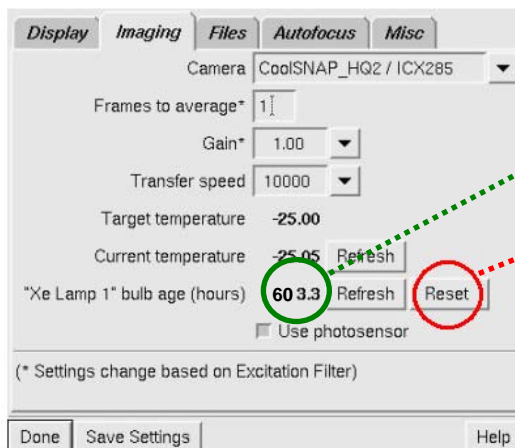
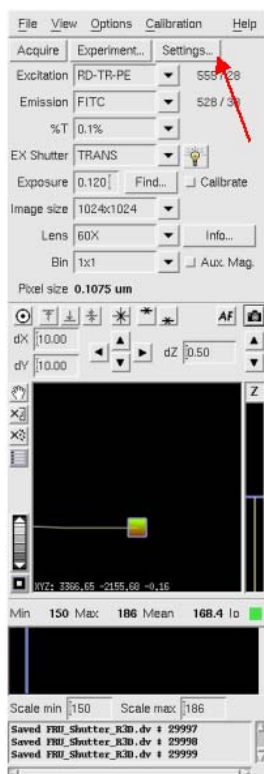
10. Tighten the two thumb screws on the end of the lamp housing.



11. Place the open end of the lamp housing over the flange on the *DeltaVision* and tighten the three hex screws as shown.



12. Turn the *DeltaVision* on as usual and start Resolve3D.
13. Before resetting the bulb age, write down the age of the bulb you just replaced. This will help you to keep track of when you may need to replace the next one.
14. Open the Imaging tab on the Settings window and reset the bulb age.

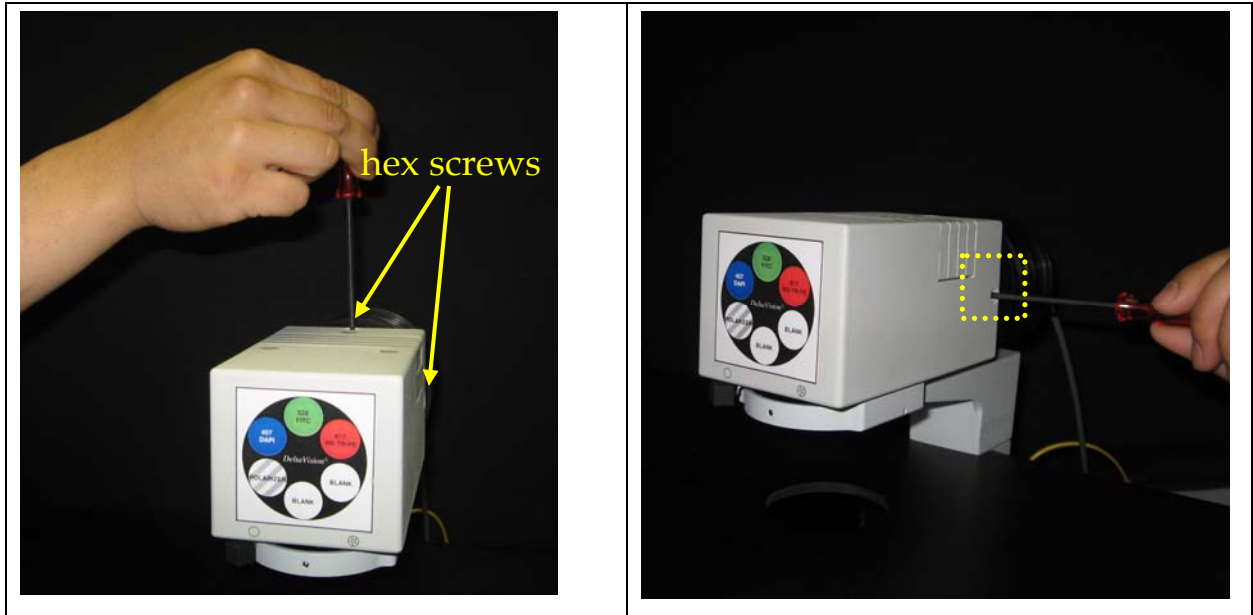


Note For *DeltaVision* systems with the Multiplexed Wavelength option installed, the procedure for changing the xenon bulb in the secondary lamp housing is identical to the procedure described above.

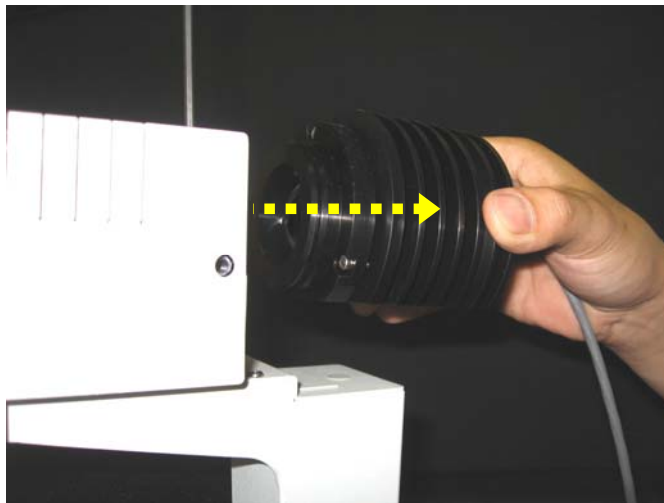
Replacing the Transmitted Light

To replace the LED transmitted light assembly:

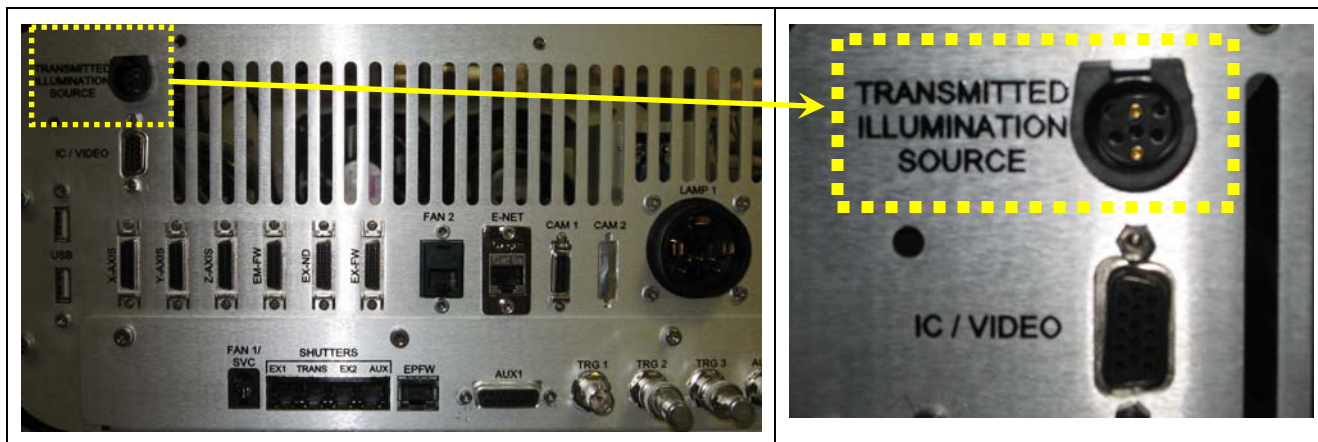
1. If the *DeltaVision* system is on, exit Resolve3D and ensure that the IC/MIC is off prior to proceeding.
2. Loosen the two hex screws on the top and right sides of the transmitted light housing as shown.



3. Carefully remove the transmitted light assembly from the housing.



4. Disconnect the other end of the attached cable from the Transmitted Illumination Source connection on the back of the IC/MIC (upper left-hand corner when facing the back of the unit; shown below).



Transmitted Illumination Source Connection (back of IC/MIC)

5. Re-insert the new transmitted light assembly (Part #52-851243-000) into the housing and tighten the two hex screws.
6. Re-connect the new transmitted light assembly cable to the Transmitted Illumination Source connection on the back of the IC/MIC (shown above).

Aligning the Illumination Path

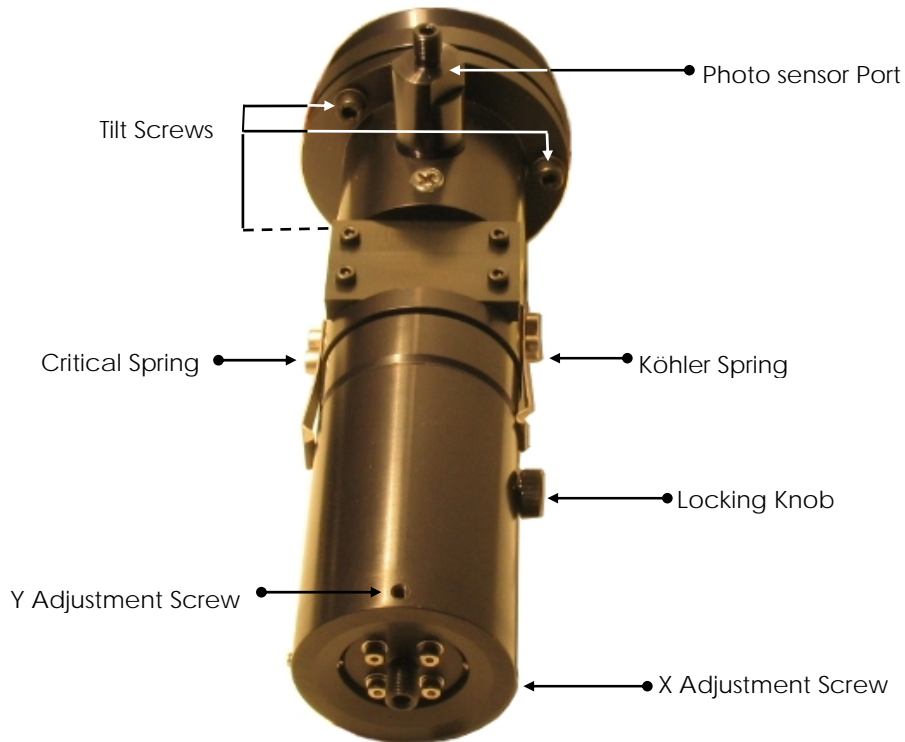
To get the best images with *DeltaVision*, make sure that the light path is aligned in both Köhler and Critical positions. Use the instructions in this section to test and adjust alignment. This section contains the following topics:

- *The Fiber Optic Module* describes the settings and adjustments on this key component. You'll use the Fiber Optic Module to switch from Köhler to Critical Illumination. You'll also use it to align the illumination path with the microscope.
- *Before You Check or Adjust the Illumination Path Alignment* lists the settings and filters that you'll need to select before you check or adjust alignment.
- *Checking Illumination Path Alignment* shows how to check the alignment in both Köhler and Critical positions. To ensure optimal performance, check the alignment every two weeks. You should also check alignment to verify that the light is reaching the sample if the images seem dim.
- *Path Alignment* shows how to align the illumination path in both Köhler and Critical positions.

The Fiber Optic Module

Use the Fiber Optic Module to align the light path from the fiber optic cable to the Fluorescence Illuminator. This module allows you to adjust the tilt, horizontal, and vertical orientation of the light path.


Figure 7: The Fiber Optic Module

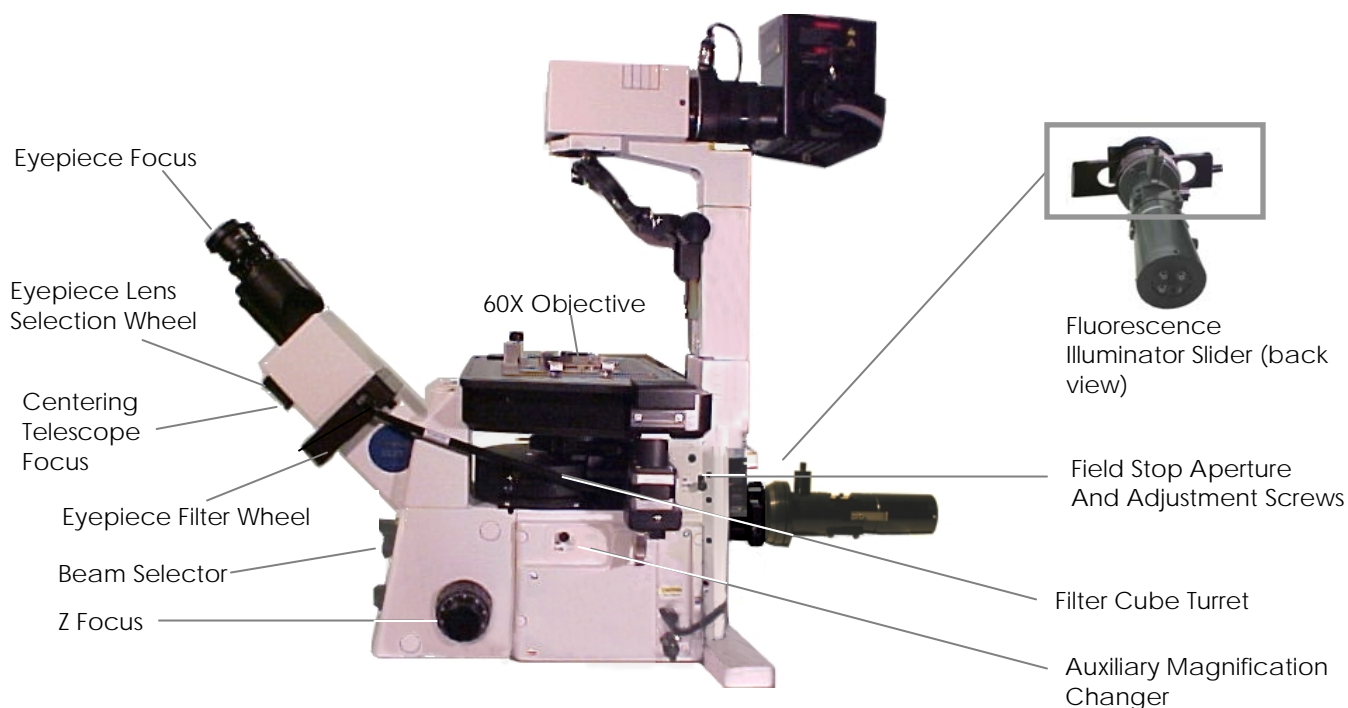


Adjustment or Lock	Use to
Tilt Screws	Adjust the tilt of the Fiber Optic Module body.
Photo-sensor Port	Connect the fiber optic cable to the photo-sensor detector in the IC/MIC.
Köhler Spring	Set the Köhler illumination position. Two locking screws hold it in position.
Critical Spring	Set the Critical illumination position. Two locking screws hold it in position.
Locking Knob	Temporarily lock the position of the Focus Control.
Y Adjustment Screw	Adjust the vertical position of the cable.
X Adjustment Screw	Adjust the horizontal position of the cable.

Before You Check or Adjust Illumination Path Alignment

Before you check or adjust alignment, remove all sources of blockage to the light path. Then verify the following settings to make sure that the various lenses and controls are in the correct positions (you can feel the controls snap into place when they are set in position).

- The Fluorescence Illuminator Slider on the back of the microscope is in an open position.
- The standard (Sedat) color set polychroic filter (usually labeled) is selected and in position on the Filter Cube Turret. (Alignment may vary between polychroic mirrors. Factory alignment is performed on the standard color set.)
- The shutter on the Filter Cube Turret is open (O).
- The Field Stop Aperture lever is pulled out all the way to close the aperture.
- The Magnification Changer is completely in (the 1X position).
- The 60X objective is in its lowest position.
- The Beam Selector is on the  (eye) position.
- The Eyepiece Lens Selection Wheel is in the open (O) position.



Checking Illumination Path Alignment

To provide an optimum light source, the tilt and position of the Fiber Optic Module must be aligned with the microscope. This procedure shows how to perform an “inside-outside” focus test to make sure that the module is properly aligned. It also shows how to perform “in focus” tests to make sure that the position of the module (distance from the microscope) is correct for Köhler Illumination, and if required, for Critical Illumination.

To check alignment:

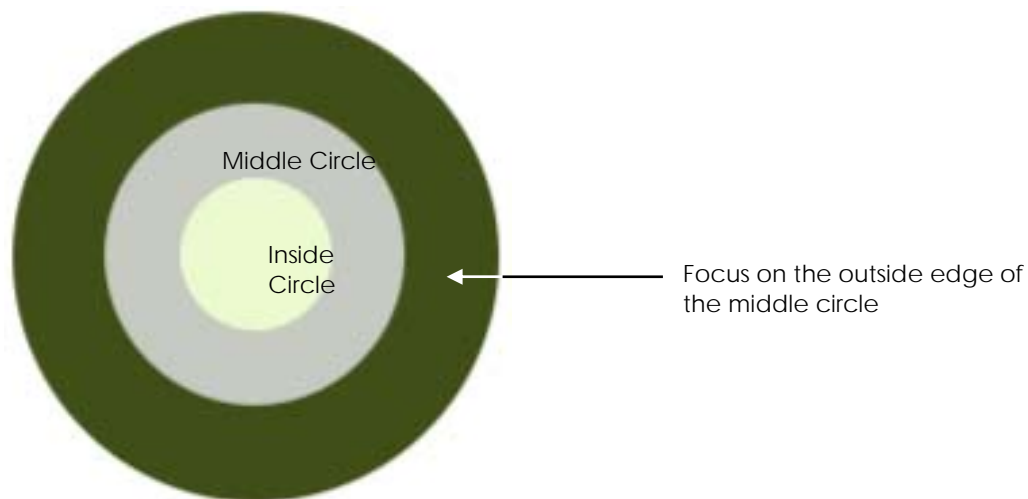
1. Move the Beam Selector to direct the light to the eyepiece and make sure that the 60X objective is lowered and in position as shown previously in

Before You Check or Adjust Illumination Path Alignment.

2. Use the Eyepiece Filter Wheel to select the FITC eyepiece filter.
3. In the Resolve3D window, select the following filters:

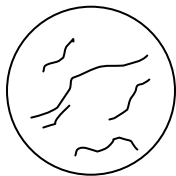
In this Field	Select
Excitation	TRITC
Emission	FITC
%T	0.1%

4. Apply immersion oil to the objective and mount the mirror slide.
5. On the keypad, press EX SHUTTER to open the Excitation shutter.
6. Use the Eyepiece Focus on the oculars to focus on scratches on the mirror slide.
7. Fully close the Field Stop Aperture and slowly rotate the fine Z Focus knob in and out of focus in both directions. The light should evenly expand around the aperture. If it flares in one direction, go to *Step One: Center the Field Stop Aperture* on Page 146.
8. Check for alignment in Köhler Illumination as follows:
 - a. Open the Field Stop Aperture. Then loosen the Locking knob and slide the focus end of the Fiber Optic Module into the Köhler Illumination position (see Page 80). Then tighten the Locking Knob.
 - b. Switch the Eyepiece Selection Wheel to the Centering Telescope position (CT). Use the Centering Telescope Focus control to focus on the objective back aperture (the outside edge of the middle circle), as shown below.



After you focus on the outside edge of the middle circle, the outside edge of the inner circle should also be in focus and the centers of the inside and middle circles should be aligned.

- c. If the image is not in focus, go to *Step Three: Set the Köhler Spring Position* on Page 149.
9. If you are using Critical Illumination, check for alignment by moving the eyepiece to the Open position (O), loosening the Locking knob, and sliding the Focus Control on the Fiber Optic Module out to the Critical Illumination position. Then tighten the Locking Knob. Both the scratches and the edge of the circle should be roughly centered and in focus, as shown below. If not, go to *Step Four: Set the Critical Illumination Position* on Page 150.



10. If you observe any alignment problems in Steps 7, 8, or 9, use the instructions in the next section to align the illumination path.

Path Alignment

This procedure shows how to align the Fiber Optic Module and the fiber optic cable with the light path. It also shows how to set the positions of the two springs that allow you to switch between Köhler and Critical Illumination.

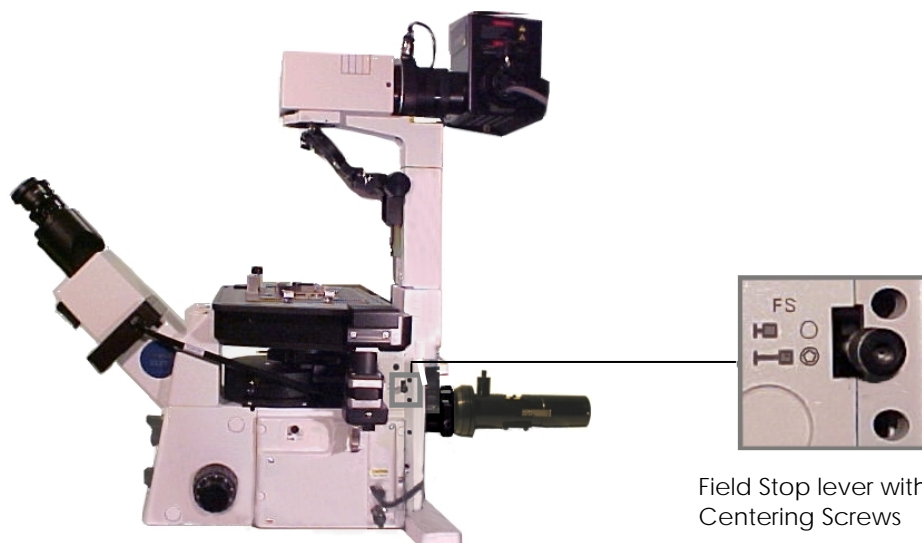
Step One: Center the Field Stop Aperture

1. Apply immersion oil to both the objective and the mirror slide and mount the mirror slide.

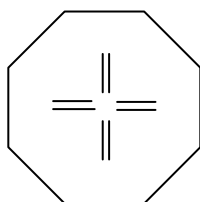
2. Use the Eyepiece filter wheel to select the FITC eyepiece filter. In the Resolve3D window, set the other filters for alignment as follows:

In this Field	Select
Excitation	TRITC
Emission	FITC
%T	0.1%

3. Press EX SHUTTER on the keypad to open the EX shutter.
4. Use the Z focus knob to focus on the scratches on the mirror slide.
5. Pull out the Field Stop lever all the way to close the Field Stop aperture.
6. Use the Olympus 3mm hex key to adjust the two Field Stop Centering Screws until the Field Stop Aperture is centered on the reticle.



The reticle should appear in the center of the octagon as shown below.



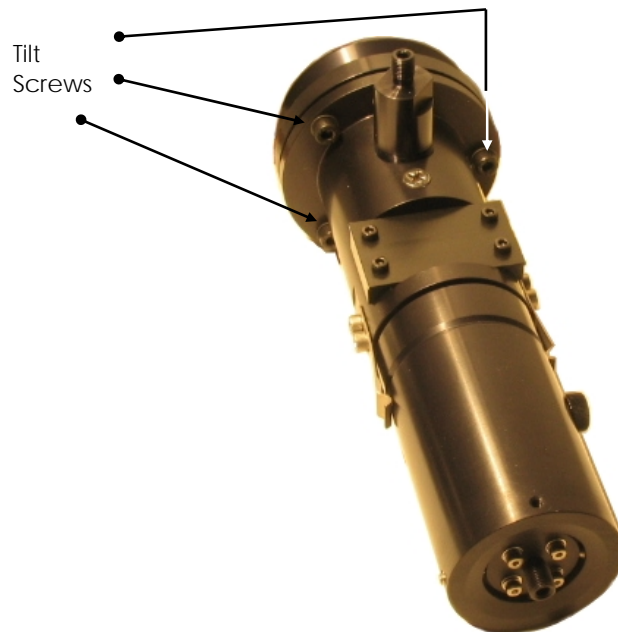
7. Fully open the Field Stop aperture.

Step Two: Adjust the Tilt of the Fiber Optic Module Body

1. Make sure that the Beam Selector on the microscope base is set to direct the light to the eyepiece and verify that the 60X objective is set in its lowest position. The Z focus knob should be turned until it stops (with the objective fully lowered).
2. Remove the oil from the objective (See *Cleaning* on Page 153) and remove the Repeatable Slide Holder.
3. Place a piece of paper on the stage with a piece of glass on top to keep it flat.
4. Press EX SHUTTER on the keypad to open the EX shutter.
5. In Resolve3D, set the neutral density filter to 100% light transmittance.
6. Pull the Field Stop lever all the way out to close it. The bright area on the paper should be circular and centered in the dim field.



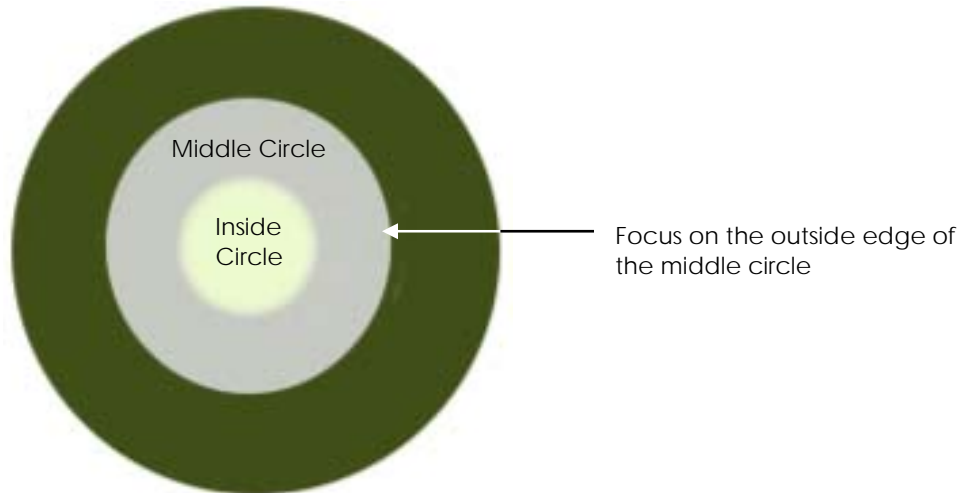
7. Use a 3 mm hex key to loosen the Tilt screws and adjust the screws so that the bright spot on the paper is circular and is centered in the dim field as shown in the previous step.



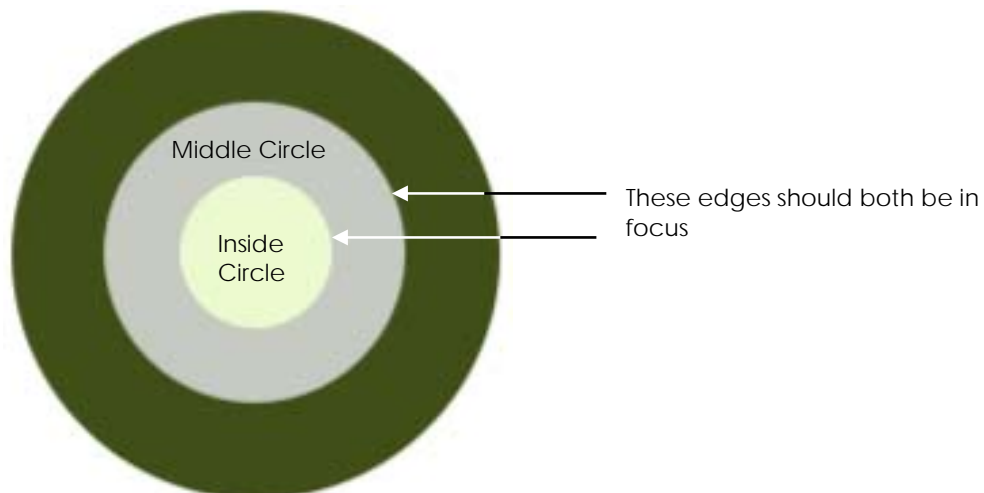
8. Tighten the Tilt screws.

Step Three: Set the Köhler Spring Position

1. Move the Eyepiece Selection Wheel from the open (O) to the Centering Telescope (CT) lens.
2. Use the Centering Telescope Focus on the ocular to focus on the objective back aperture (the outside edge of the middle circle).



3. Loosen the Locking Knob and use a 2.5 mm hex key to loosen the two Köhler Spring screws. Then move the Focus Control back and forth until the edge of the inner circle is in focus. After you focus the edge of the inner circle, the edges of both the inner and middle circles should be in focus.

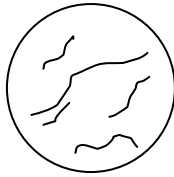


4. Holding the Focus Control still, tighten the Locking Knob.

5. Slide the Köhler Illumination spring so that it is in the groove on the Focus Control and tighten the Köhler spring locking screws to set the Köhler position.

Step Four: Set the Critical Illumination Position

1. Move the Eyepiece Selection Wheel to Open (O) and fully open the field aperture.
2. Apply immersion oil to both the objective and the mirror slide and mount the mirror slide.
3. Loosen the Locking Knob. Then move the Focus Control on the Fiber Optic Module out approximately 5 mm and then slide it back and forth until the edge of the circle snaps into a sharp focus. You should see both the scratches and the edge of the circle in focus as shown below.



Note Critical Illumination is typically 5mm out from Köhler Illumination.

4. Tighten the Locking Knob.
5. Check the focus. If the edges on the circle are not crisp, loosen the locking knob, re-adjust the Focus Control, and tighten the Locking Knob again.
6. Slide the Critical Illumination spring into the groove on the Focus Control and tighten the Critical Illumination spring screws with a 2.5 mm hex key.

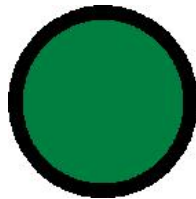
Step Five: Adjust and Test the Alignment

1. Fully close the Field Aperture and slowly rotate the microscope Z focus knob in and out of focus. The light should evenly expand around the aperture.
 - a. If the light does not expand evenly around the aperture, make sure that:
 - The shutter is open.
 - The Eyepiece Selection Wheel is set to 0.
 - The mirror slide is mounted in immersion oil and in focus.
 - The Fiber Optic Module is in Critical position (see Step 3).
 - b. Use a 1.5 mm hex key to adjust the X and Y adjustment screws on the top and left sides of the Fiber Optic Module until the image is centered in the reticle as shown below:



2. Adjust the center alignment in the Köhler position as follows:

- a. Slide the Focus Control to the Köhler position, set the eyepiece to **CT**, and use the Centering Telescope Focus to focus on the objective back aperture. You should see the area between the back aperture of the objective lens (the darker area) and the fiber tip (bright green).

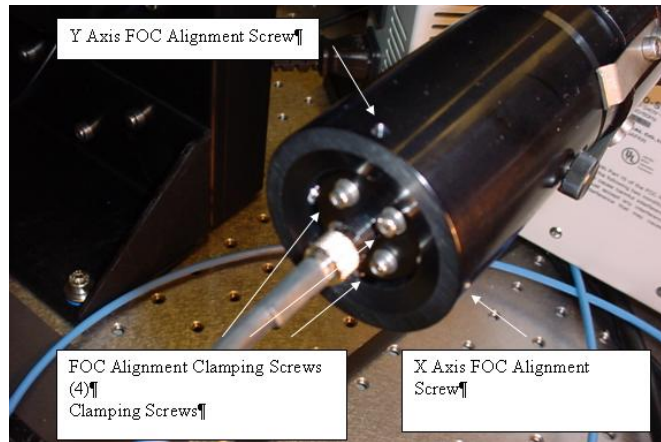


- b. Use a 1.5 mm hex key to adjust the X and Y adjustment screws on the top and left sides of the Fiber Optic Module until the fiber tip image is at a point that is midway between its original center and the actual center (the point where the centers of the two circles are aligned).
3. Adjust the tilt of the Fiber Optic Module as follows:
- a. Make sure that the viewing knob on the microscope base is set to direct the light to the eyepiece and verify that the 60X objective is in detent and in its lowest position.
 - b. Remove the oil from the objective.
 - c. Place a piece of paper on the stage with a piece of glass on top to keep it flat.
 - d. Press EX SHUTTER on the keypad to open the EX shutter and set the neutral density filter to 100% light transmittance (in Resolve3D).
 - e. Use a 3 mm hex key to adjust the X and Y tilt screws so that the bright spot on the paper is circular and is centered in the dim field.
4. Repeat steps 1-3 in this procedure until the image is centered in both the Critical and Köhler positions.



Note Aligning for Critical and Köhler illumination is an iterative process. The alignment will improve with each iteration.

5. When the image is centered, tighten the clamping screws on the Fiber Alignment Disk.



6. Fully close the Field Aperture and slowly rotate the microscope Z focus knob in and out of focus. The light should evenly expand around the aperture. If the light tends in one direction, call the API Customer Service Hotline (1-800-862-5166).
7. Move the Fiber Optic Module between the Köhler and Critical position several times and make sure that the positions are repeatable (within about 1%). If the Köhler and Critical positions are not repeatable, call the API Customer Service Hotline (1-800-862-5166).

Replacing IC/MIC Fuses

Follow these instructions to replace the fuse in the Microscope Interface Chassis. To replace fuses for other components, follow the instructions in the manuals that are provided for those components.



CAUTION: Installation of improperly rated fuses can cause damage to the system.

To replace a fuse:

1. Shut down the system.
2. Unplug the power cord on the back of the IC/MIC.
3. Remove the fuse holder.
4. Test the fuses with a continuity meter.
5. Replace any bad fuses with 5X20mm 6.3A 250V UL high break capacity fuses (API P/N 19-170045-000).
6. Install the fuse holder.
7. Plug in the power cord.

Cleaning

Most system surfaces are best cleaned with a lint-free cloth or lint-free swabs and spectroscopy-grade isopropyl alcohol or chloroform. Avoid contaminating the cleaning solution by never reusing the cleaning cloth or swabs. Operators should be trained in the handling of flammable liquids such as alcohol. Material Safety Data Sheets (MSDS) should be maintained for the cleaning solutions, as with any hazardous material.

The exceptions to this cleaning practice are the polychroic mirror and the optical filters. These components should be cleaned with low-pressure air. For example, use a bulb designed for cleaning camera lenses, which blows air across the surface. Do not use high pressure. Do not use canned air, as this often leaves a fluorescent residue.

To clean the microscope, follow the instructions in the manufacturers' manuals that are provided for these components.



CAUTION: Improper cleaning of the polychroic mirror and the optical filters will result in damage.

Moving the System

If you need to move your *DeltaVision* system, call Applied Precision for instructions.

A

Appendix A: The Immersion Oil Kit

The immersion oil kit is a collection of oils with refractive indexes that range from 1.500 to 1.534. Use of the correct immersion oil decreases the spherical aberration in the image data.

- For *DeltaVision Core*, the immersion oil kit includes eighteen oils that range from 1.500 to 1.534, in increments of 0.002.
- For *personalDV*, the immersion oil kit includes six oils that range from 1.512 to 1.522, in increments of 0.002.

Many factors influence the optimum refractive index of the immersion oil, including specimen preparation, temperature, humidity, and atmospheric pressure.

The Oil Calculator

In order to calculate the desired refractive index, *softWoRx* is equipped with the Lens Information function. This function is found in the Utilities menu in *softWoRx*. It can also be accessed from Resolve 3D by clicking the **Info** button. The following parameters are explained here to help you enter the appropriate information and use the resulting calculations.

Distance from Coverslip to Specimen (microns)

Establishes the distance from the surface of the coverslip to the desired focal plane.

Temperature

Defines the temperature of the specimen and the immersion medium.

Specimen Refractive Index

Defines the refractive index of the specimen, which is usually that of the mounting medium. In some cases, the specimen itself contributes significant refraction.

Recommended Refractive Index

Displays the resulting optimal refractive index of the immersion oil. Actually experimenting with oils with refractive indexes very close to this value is the best way to select the optimal oil.

Resolution Ratio

Displays the ratio between the Z resolution and the XY ratio. This serves as a reference to the degree of Z elongation.

Maximum XY Pixel Size

Displays the maximum recommended XY pixel size for deconvolution.

Recommended Z Step

Displays the smallest possible Z step for this objective. Choosing a smaller Z step will add to the size of the image file but will not improve image quality.

B

Appendix B: Troubleshooting

This appendix was designed to help you diagnose and correct the most common problems encountered on the *DeltaVision* system. It covers two types of troubleshooting tasks:

- Diagnosing System Problems
- Analyzing Reasons for Poor Image Quality

If you are unable to correct a problem, fill out the *DeltaVision* Problem Report Form at the end of this appendix and either e-mail it to hotline@api.com or fax it to 425-557-1055, attn: Bio Service Hotline.

Diagnosing System Problems

Troubleshooting the Controller

The following table shows the most common Instrument Controller problems and their resolutions.

Table B-1: Controller Troubleshooting Chart

Indication	Cause	Correction
Encoder Error when initializing stage.	Poor cable connection.	Power down system, including IC/MIC. Reseat X, Y, and Z motor cables. Reseat motor cables on excitation module. Power up system.
Stalled sequence of images.	Computer misallocated memory.	Exit the software and restart IC/MIC.

Troubleshooting the Workstation

Other system troubles are indicated by messages or readings in the software. The Resolve 3D message window displays Resolve 3D activity. Observe the messages in this window when troubleshooting. This table shows possible problems and corrective actions.

Table B-2: Workstation Troubleshooting Chart

Indication	Cause	Correction
"File system full" message when trying to save images.	There is no more storage space for image data.	Delete unwanted files. Save image files to CD or DVD or LAN. See softWoRx Imaging Workstation User's Guide for more information.
"Camera not found" message.	Power up sequence was incorrect.	Shut down the system and then restart it using the steps described in <i>Chapter 9: Maintenance</i> .
Resolve 3D settings are not updating changes made using the keypad or joystick (for example, the filter selection, or Z position).	Lack of communication between the Instrument Controller and Workstation.	Shut down the system and then restart it using the steps described in <i>Chapter 9: Maintenance</i> .
At login, user name not recognized.	User has not been added.	Add user. See the softWoRx Imaging Workstation User's Guide.

Analyzing Reasons for Poor Image Quality

The following table documents the most common acquisition difficulties and abnormalities in image data.

Table B-3: Image Quality Troubleshooting Chart

Indication	Cause	Correction
Dim images or long exposure times.	Poor illumination.	Fully open field aperture. Align xenon lamp and fiber optic cable as shown in Replacing the Xenon Bulb

Indication	Cause	Correction
		<p>on Page 136.</p> <p>Ensure that filter cube turret is locked in position on rail mount.</p> <p>Ensure shutter on filter cube is open.</p> <p>Ensure slider behind microscope is seated in an open position.</p> <p>Ensure proper filter cube is in position and seated in detent.</p>
Dim illumination. When fiber optic cable and focusing lens are removed, the projected light does not form a circle.	Filter wheel(s) out of alignment.	<p>Shut down and start up as described on Pages 17 and 28 or unplug and plug in the Eyepiece filter wheel. This will reset the home position of the filter wheels and align filter wheel position.</p> <p>Calibrate filter wheels following instructions on Page 121.</p>
Dark, out of focus spots on image.	Dust interference.	Clean polychroic filter, emission filter, and camera window using low-pressure air. Do not use canned air. See Page 153 for further recommendations regarding cleaning system components.
Image is distorted around edges or throughout. Occlusion seems to creep in toward center.	Condensation on camera window, possibly due to improper camera temperature.	<p>Clean camera window using low-pressure air. Do not use canned air. See Page 153 for further recommendations regarding cleaning system components.</p> <p>Check camera temperature in Resolve3D. Consult camera documentation for proper setting.</p>
Brightness of Z section images varies greatly	A broken Photo sensor cable	<p>Disconnect the Photo sensor cable and the EX module cable.</p> <p>Connect the Photo sensor cable to the EX module.</p> <p>Set the Excitation filter to FITC or some other visible light.</p> <p>Open the EX shutter.</p> <p>Bend the cable and examine it for light leaks. If you observe a light leak, replace the cable.</p> <p>Direct the light to a wall. If you observe inconsistencies in the light output as you bend the cable, replace the Photosensor cable.</p>

Table B-3: Image Quality Troubleshooting Chart Cont'd

Indication	Cause	Correction
Image has a traveling light or bubble.	Air bubble in immersion oil.	Clean front and back surfaces of objective and coverslip. Reapply immersion oil and restart experiment.
Interference in image data.	Possibly dirt, dust, oil, or air bubble.	Clean front and back surfaces of objective and coverslip.
Very bright image or camera saturation message.	Camera saturation.	Use lower exposure time and/or higher neutral density filter.
Z series shows uneven or off center illumination.	Poorly aligned illumination.	Align xenon lamp and fiber optic cable. See Chapter 9, Maintenance.
No image when Acquire is pressed.	Knob at base of microscope is directing light to the eyepiece.	Move knob to direct light to camera.
Z series out of focus and incomplete.	Stage was not centered within sample at start of experiment.	Position stage in center of the sample and run experiment again.

DeltaVision Problem Report Form

Research Facility:	System Serial Number:
Contact Person:	softWoRx version: (use <i>Help</i> → <i>Software Versions</i> to display)
Phone:	
E-mail:	Date:
Problem Encountered: Please write a detailed description, answering as many of the following questions as possible.	
Questions:	
1	When did this first occur?
2	Was there any recent change or update to the system prior to the problem occurring?
3	What sequence of operations produces the problem?
4	What other programs were running when you encountered the problem?
5	What error messages, if any, were shown?
6	Is the failure the same each time or does it show different symptoms?
7	Does it occur consistently or is it random?
8	Does it go away after the workstation is re-booted?
9	Does it go away after the instrument controller is re-booted?
10	How often do you re-boot the workstation and instrument controller?
Additional Comments:	
(See Page 2 for additional clarification issues)	
Please supply the following log files:	
Workstation: (/home/userName/softworx-logs/softworxlog.txt)	
Instrument Controller: (c:\ic530_dv\log\IC_530.log or c:\ic525_dv\log\IC_525.log or c:\ic540_dv\log\IC_540.log)	

Please e-mail this form to hotline@api.com or Fax to: 425-557-1055, attn: Bio Service Hotline

C

Appendix C: Acquiring a PSF

This appendix shows how to acquire a Point Spread Function (PSF) and convert it to an Optical Transfer Function (OTF).

- *Acquiring a PSF* shows how to measure a Point Spread Function.
- *Converting PSF to OTF* shows how to convert the Point Spread Function to the Optical Transfer Function that is required to process images.

Before You Start

Before you attempt to measure a PSF, check the OTF library that is included with *softWoRx* to find out if the library provides an OTF for your objective.

Acquiring a PSF

To measure the point spread function (PSF) you need to optically section a fluorescent bead. Since the properties of the objective lens are the most important elements of determining a PSF, it is necessary to measure the PSF whenever new lenses are added to your microscope. The deconvolution software adapts to the PSF (actually the OTF) wavelength, so it is unnecessary to measure the PSF at more than one wavelength.

Note If you do not have the tools necessary to acquire a PSF, *softWoRx* includes a utility that allows you to calculate a theoretical OTF based on the numerical aperture of the camera lens, index of refraction, and emission wavelength. If the aperture of the lens is lower than 0.75 N.A., the calculated OTF may work as well, or even better, than a measured OTF; however, if the aperture is greater than 0.75 N.A., a measured OTF will generally give you better results. For information about calculating an OTF, see the *softWoRx* online Help.

A well-measured PSF is a key to successful deconvolution. For this reason, make sure that you:

- Thoroughly check all imaging conditions.
- Take the time you need to get a good signal-to-noise ratio in the image.
- Completely scan the bead.

Tools

This procedure requires the following tools:

- A clean and aligned 3-D microscopy system
- A bead slide with 0.1 μm , or smaller, fluorescent beads
- A grid slide or other microscopic ruler
- An immersion oil set, if appropriate (see
- Selecting the Correct Immersion Oil on page 167.)

The following steps describe how to calculate pixel size, measure the PSF, and obtain the corresponding OTF.

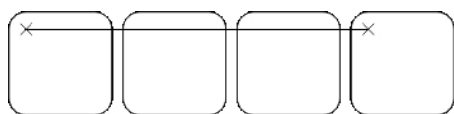
To calculate pixel size:

1. Place the new objective in the Objective Turret and set the Max image size, best camera speed, and magnification slider at 1X.
2. Use the Eyepiece Filter Wheel to select the FITC eyepiece filter.
3. In the Resolve3D window, select the following filters:

In this Field	Select
Excitation	TRITC
Emission	FITC
%T	0.1%

4. Place the silicon target grid on the stage and focus it (9.995 μm /square). Align the grid image to the vertical and horizontal axis and maximize the image.

5. Switch the beam selector to **SP** or **SPL**. Then adjust the exposure time and %T filter and click **Acquire**. Leave Data Collection window 21 open.
6. In the Image window, choose **Tools | Measure Distance**. Then set the **Units** to Pixels in the Measure Distance dialog box.
7. Draw a line across the image from a point on the top left square to a point in the same relative position on the top right square.



8. If the vertical delta is more than four pixels, re-align the slide. Repeat this process at the middle and bottom. Then count and record the number of grid elements and record the distance in pixels.
9. Repeat Steps 7 and 8 in the vertical direction.
10. Calculate the pixel size for each of the six measurements (top, middle, bottom, left, center, and right) as follows:

$$\text{Pixel Size(um)} = \frac{9.995 \text{ um/box}}{\text{Sum of (Measured pixels)/(Number of grids per measurement)}}$$

Your calculation should be accurate to four decimal places.

11. Average the six pixel sizes to obtain the correct pixel size.

To acquire a PSF:

1. Obtain the objective lens ID number (see the *softWoRx* online Help for information about lens identification numbers).
2. From the softWoRx main menu, choose **Utilities | Revise Microscope Configuration**. Then enter the root password to open the RESOLVE3D.SYS file.
3. In the RESOLVE3D.SYS file, under the Microscope Specifications section:
 - a. Increase the number of lenses next to MS_Number_Lenses: by 1.
 - b. Add the name of the objective to MS_Lens_Names: (e.g., 100Xoil, 60Xwater).
 - c. Add the lens ID to MS_Lens_ID_Numbers:
 - d. Enter the pixel size for the new lens.

For example:

If the desired lens is 40X/1.35 with ID=10403 (the third lens in the list), then the pixel size is 0.1656.

MS_Lens_Names: 10X 20X 40X 60X 100X

MS_Pixel_Sizes: 0.6680 0.3313 0.1656 0.1103 0.06631

MS_Lens_ID_Numbers: 10105 10205 10403 10602 10002

4. Mount a bead slide on the microscope and focus on the beads to obtain the maximum intensity. Find a bead that is located by itself. (Refer to "Finding Beads" located immediately after this procedure.)
5. Use the Center Object tool to center a single bead in the X and Y directions. (It is helpful to collect large images, such as 1024×1024.)
6. Adjust the CCD exposure time so that the maximum intensity at the plane of best focus is at least 2000 counts. Make sure that the camera does not saturate at the plane of best focus.
7. Now use a 256×256 image.
8. Ensure there is only one bead in the field of view and that, as you go out of focus, no rings from other nearby beads enter the image.
9. Verify that your microscope and software are accurately configured for lens and auxiliary magnification.
10. Execute the Standard PSF Measurement Macro described in the online Help to measure the standard point spread function (or run a Z series through the bead consisting of 128 sections acquired in 0.1 μm Z increments).
11. Run the *softWoRx* PSF to OTF program that converts the optical sections into an OTF. (Refer to *Converting PSF to OTF* later in this chapter.)


Finding Beads

It is easiest to find beads in a very dark room. Bead slides from Applied Precision include 1 μm beads that fluoresce brightly at 617 nm and 0.1 μm beads. Coarsely focus on the slide by positioning the lens near the slide. Scan the slide while looking for fluorescent haze from the 1 μm beads. When you focus on the fluorescence haze from the 1 μm beads you should also find the 0.1 μm beads.

Note Although a replacement bead slide is included in the Slide kit, bead slides have a limited shelf life. To purchase bead slides from Applied Precision, contact us at the appropriate number or address listed in *Chapter 1: Getting Started*.


Selecting the Correct Immersion Oil

Accurate PSF measurements depend on the selection of the correct immersion oil. Our experience has shown that the oils recommended by microscope manufacturers are often not ideal for 3-D microscopy. We recommend that PSFs are measured with a minimal amount of spherical aberration. Inappropriate immersion oils yield asymmetric PSF measurements as a result of spherical aberration. In the case of Olympus and Zeiss microscopes, an index of refraction equal to 1.518 is ideal for measuring beads that are mounted in glycerol using 1½ coverslips. Nikon microscopes use an index of refraction equal to 1.512. There are many variables that can affect the selection of the correct immersion oil. The *softWoRx* Lens Information program can help you select the proper oil.

 **Note** An oil kit is included with your *DeltaVision* system. To purchase replacement oil, please contact Applied Precision.

To confirm that you are selecting the correct immersion oil:

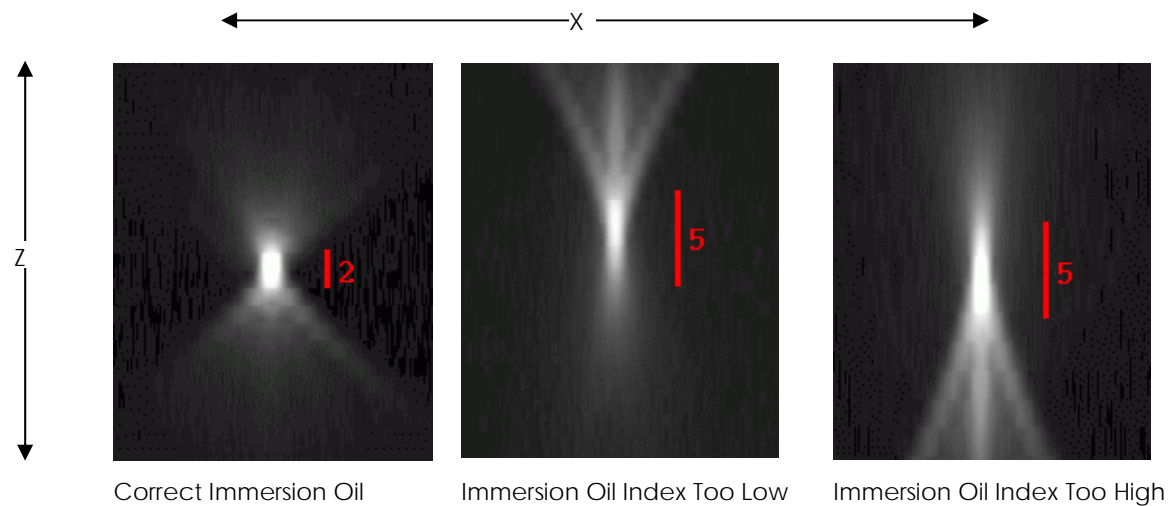
1. Collect the image data and generate a 3-D maximum intensity volume projection.
2. Rotate the 3-D image to get a view of the XZ or YZ plane. For example, rotate the image 90 degrees about the X axis or 90 degrees about the Y axis. To better see the shape of the PSF, it is helpful to do an exponential scaling—an exponent of .5 usually works well.

 **Note** If you are using *softWoRx*, use the Volume Viewer to generate a 3-D rendering of the bead scan.

3. Look for symmetric flare in the resulting image. Symmetry indicates that the oil is correct, and in virtually all situations, the most symmetric PSF along the Z axis is also the smallest and has the highest intensity. In other words, symmetry corresponds with the highest resolution.
4. Repeat the process with different oils until you determine the optimal immersion oil.

The following figure demonstrates how image flare can be affected by the use of different immersion oils.

Figure C-1: Flare from Immersion Oils (3-D Maximum Intensity Projections)



Converting PSF to OTF

The PSF to OTF program converts a measured point spread (PSF) to an optical transfer function (OTF). Essentially, the OTF is the Fourier transform of the PSF. The pixel size of the resulting image is given in cycles/ μm . To reduce problems associated with measurement noise, the PSF is radially averaged during the conversion and, as a result, the 3D PSF image becomes a 2D OTF image.

The horizontal axis of the OTF represents axial (Z) frequency and the vertical axis represents radial (XY) frequency. The brightness of the OTF image elements, on a scale of 0 to 1, represents the frequency response of the microscope system at the corresponding radial and axial frequencies.

Figure C-2: Sample OTF Image

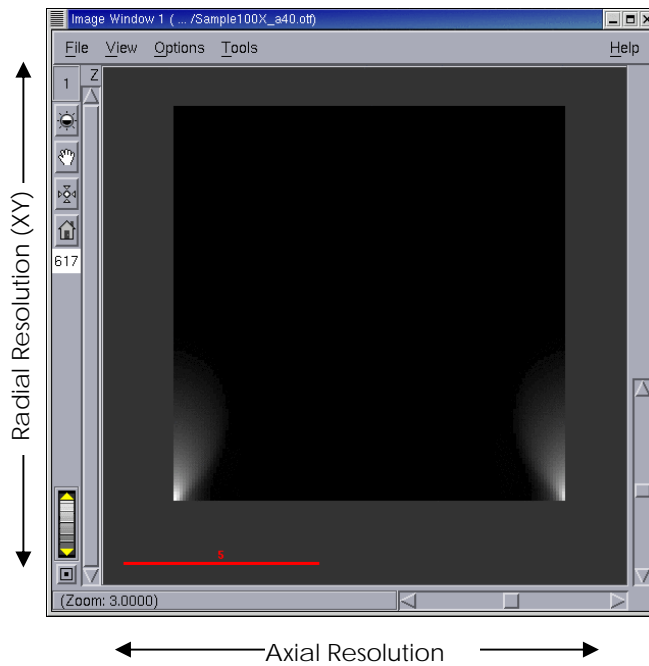
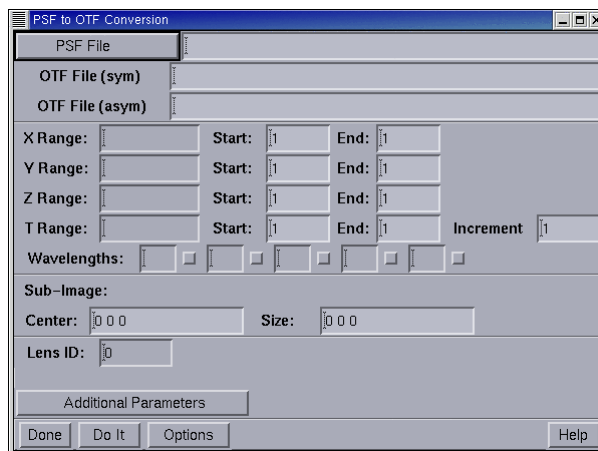


Figure C-3: PSF to OTF Conversion



Each option in PSF to OTF Conversion is described briefly below. For additional information regarding these options, refer to the online Help.

PSF File

Defines the name of the PSF image file to be converted to an OTF.

OTF File (sym)

Displays the name of the resulting axially symmetric OTF. (For your convenience, the OTF filename is created by appending “_otf” to the PSF filename.)

X Range

Defines the start and end pixel numbers in X.

Y Range

Defines the start and end pixel numbers in Y.

Z Range

Defines the start and end pixel numbers in Z.

T Range

This field is not used for PSF to OTF conversion.

Wavelengths

Determined by PSF wavelength.

Lens ID

Specifies the lens identification number (e.g., 12004).

Sub-Image: Center

Specifies the central XYZ coordinates of the point spread.

Sub-Image: Size

Specifies the XYZ image dimensions about the central coordinates. (The standard *softWoRx* point spread measurement is 256×256×128.)

Additional Parameters: Border Rolloff (voxels)

Specifies the number of voxels to rolloff at the edge of the image. This reduces edge effects resulting from the Fourier Transform used in the PSF to OTF conversion.

The procedure for converting a PSF to an OTF is very simple. After the PSF file has been identified in PSF to OTF Conversion, *softWoRx* assigns default settings to the rest of the options in the dialog. In almost every instance, these settings will be appropriate to use for the conversion.

To convert a PSF to an OTF:

1. Click **Conversions** on the main menu bar of *softWoRx*.
2. Click **Convert PSF to OTF** in the **Conversions** menu. PSF to OTF Conversions will appear.
3. Use one of the following options to enter the PSF file to convert.
 - Drag the appropriate PSF file from the File Manager into the **PSF File** text box.
 - Click **PSF File** to display a small version of the File Manager and then choose the PSF file that you wish to convert.

- Type the desired path and filename into the **PSF File** text box.

4. Click **Do It**.

To place OTF into OTF Library

If the objective used is in addition to those already present, you'll need to modify *softWoRx* to use the new objective by adding the file to either */usr/local/softWoRx* or *dv2.10/config/system.dvrc* as follows:

1. Log in to Linux as root.
2. Navigate to */usr/local/softWoRx/config/system.dvrc*
3. Find the section labeled, "Lens-to-OTF matching" and follow the instructions provided for the OTF file.

The following is an example of this section of the file:

```
# Lens-to-OTF matching. These are of the form
LENS_<lensIDNumber>_OTF
# and are defined to be the file name in the OTF directory
of the OTF that is
# to be used for this lens ID.
LENS_12_OTF           60X140_sample.otf
LENS_10602_OTF        60X140.otf
LENS_10003_OTF        100X135.otf
LENS_10403_OTF        40X135_sample.otf
LENS_10603_OTF        60Xw_120.otf
LENS_10205_OTF        20X0.75c.otf
```

D

Appendix D: Reference Information

The appendix includes the following topics:

- Standard Filename Extensions lists the filename conventions used by DeltaVision.
- Standard Fluorescence Filters shows the excitation and emission peaks of the standard filters included with DeltaVision.
- Live Cell Filter Sets shows the excitation and emission peaks of the filters that are included in the optional Live Cell filter wheel module.
- Reference List includes references for microscopy, Linux, image processing, optics, microscopy, and sample preparation.

Standard Filename Extensions

The following is a list of filename conventions used by *DeltaVision*.

Filename Extension	Type of File
*.dv	Standard <i>DeltaVision</i> image
*.otf	Optical Transfer Function
*_R3D.dv	Resolve3D image
*_D3D.dv	Deconvolved image
*_VOL.dv	Volume Rendered image

Standard Fluorescence Filters

DeltaVision provides a four color filter set, a polarizer, and multiple sets of optional filters. These filters are designed to be used with many common fluorescent probes. If you are using fluorescent probes that are not well matched with the standard *DeltaVision* filters, contact Applied Precision for assistance.

The excitation and emission peaks of the *DeltaVision* filters² are provided in the following table.

Filter Name	Excitation	Emission	Appropriate Probes
DAPI	UV, 350nm	Blue, 455nm	DAPI, Hoechst, Coumarin
FITC	Blue Green, 490nm	Green, 525nm	Fluorescein, GFP, CY3
TRITC	Green, 555nm	Orange, 605nm	Rhodamine, Texas Red, Phycoerythrin,
CY-5®	Red, 645nm	Infrared, 705nm	CY-5
CFP (optional)	Deep Blue, 436 nm	Blue Green, 470nm	CFP
YFP (optional)	Blue Green, 500 nm	Yellow Green, 535nm	YFP

Live Cell Filter Sets

The optional Live Cell filter wheel module includes four of the most common sets of filters used for live cell imaging.

Filter Name	Excitation	Emission	Appropriate Probes
CFP	Deep Blue, 430nm	Blue, 470nm	Cyan GFP (CFP)
YFP	Blue Green, 500nm	Yellow Green, 535nm	Yellow GFP
mCherry	Yellow, 572nm	Red, 632nm	DsRed Express, DsRed2
EGFP	Blue, 470nm	Green, 525nm	EGFP, sgGFP

² Data for filters provided by *Chroma Technology Corp.*

Reference List

Selected references are provided on the following pages. Contact Applied Precision for the most recent list. If you notice omissions from the list, please inform Applied Precision.

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E

Appendix E: Resolve3D and Keypad Options

This chapter describes the following Resolve3D windows and dialogs:

- *The Resolve3D Window* is the main window for data acquisition.
- *The Design/Run Experiment Dialog* provides tools to select, design, edit, and execute experiment macros.
- *The Experiment Designer* is used to generate experiment command macros.
- *The Settings Dialog* used to control how images are displayed, select camera settings, and specify file output.
- *Keypad/Joystick Operation* is a reference for the buttons on the keypad. Many of the Resolve3D functions are also available on the keypad and joystick.

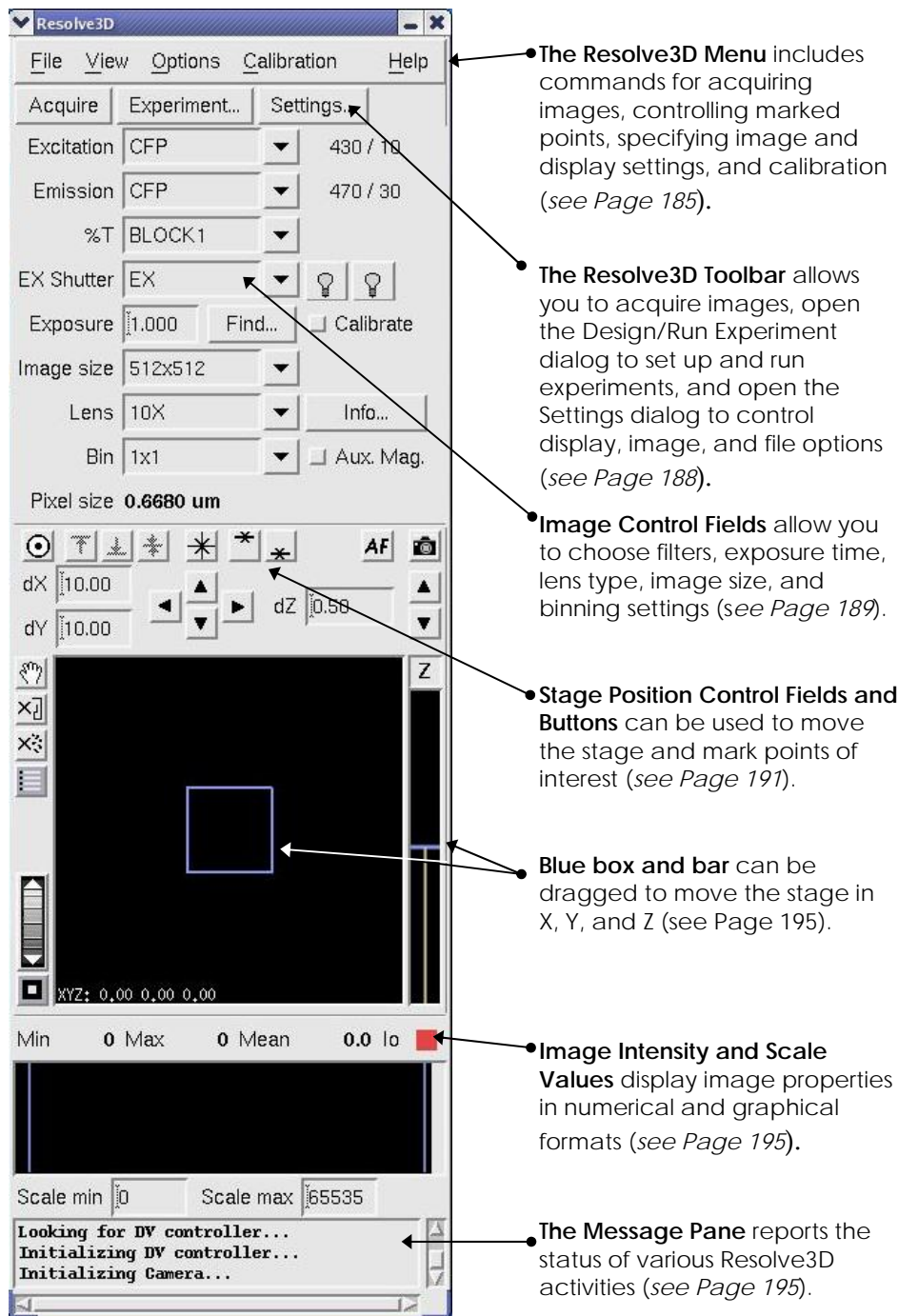
The Resolve3D Window

The Resolve3D window is the main data acquisition window. In addition to providing many of the acquisition options and controls, it provides access to the other dialogs and windows that are used for data acquisition.

To open Resolve3D:

- From the *softWoRx* menu, choose **File | Acquire (Resolve3D)**.

Figure E-1: Resolve3D Window



The Resolve3D Menu

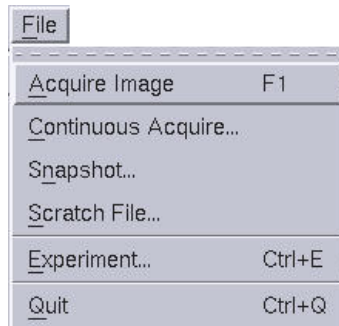
The Resolve3D menu has the following menu items:

- **The File menu** includes commands to acquire images and to open the key dialogs for setting up and running experiments.
- **The View menu** includes commands to manage marked points and to create a blank screen.
- **The Options menu** includes commands to open the Settings dialog box (where you can set display and image options) and to save settings.
- **The Calibration menu** opens the Calibration tool.
- **The Help menu** provides options to show or hide ToolTips and to get Help.

The File Menu

Use the following Resolve3D File menu commands to acquire images, create scratch files, and open the Design/Run Experiment dialog box.

Figure E-2: The Resolve3D File Menu



Acquire Image

Collects and displays an image from the microscope. This image is only displayed in the Data Collection window. It is not saved to a disk file for later use.

Continuous Acquire

Opens the Continuous Acquire dialog box that you can use to collect and display images continuously. These images are only displayed; they are not saved to a disk file.

Snapshot

Launches a tool to let you collect a single 2-D, multi-wavelength "snapshot" image. If you need to collect a Z series, time-lapse image, or other complex scheme, you will need to design an experiment with the Resolve3D Experiment Designer.

Scratch File

Creates a "scratch file" to which you can save individual image frames for later use. After a file is opened, clicking **Save Current Image** saves the most recently collected image frame. Clicking **Close Scratch File** or **Done** closes the file. Note that the image size cannot be changed while a scratch file is open.

Experiment

Opens the Design/Run Experiment dialog box. You can use Design/Run Experiment to select a previously created experiment, open the Experiment Designer window to design a new experiment, or open the Experiment Macro Editor to create or edit an experiment macro.

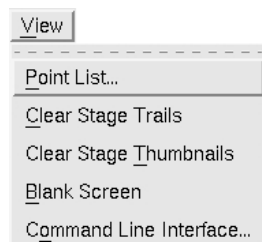
Quit

Closes the Resolve3D window.

The Resolve3D View Menu

Use the View menu to manage points that you have marked, to clear the history of the path that you took while exploring your sample, or to create a black screen for light-sensitive conditions.

Figure E-3: The Resolve3D View Menu



Point List

Opens the Points List dialog box. This dialog helps you manage a list of points (with X, Y, and Z coordinates) that you want the system to remember. These points can be interactively "visited" at any time and they can be used in experiments.

Clear Stage Trails

Clears the Stage Trails history. (The system maintains a history of the paths of motion that you take while exploring your sample. These paths are displayed as "Stage Trails" on the Stage View.)

Clear Stage Thumbnails

Clears all of the thumbnail images currently displayed on the stage view.

Blank Screen

Turns the computer screen blank. This is useful when you are imaging under very light-sensitive conditions. Clicking anywhere on the screen restores it.

Command Line Interface

Opens the Command Line dialog box that provides advanced users with the ability to issue individual Resolve3D commands to the system.

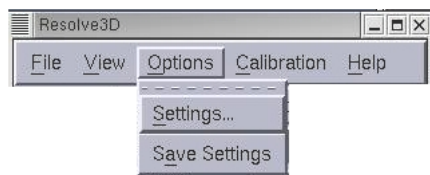


CAUTION: The Command Line Interface should be used carefully because it can put the system in an unstable state.

The Resolve3D Options Menu

Use the Options menu to open the Settings dialog box (where you can set display and image options) and to save configuration settings and state information.

Figure E-4: The Resolve3D Options Menu



Settings

Opens the Settings dialog box that allows you to control display, imaging, and file output options.

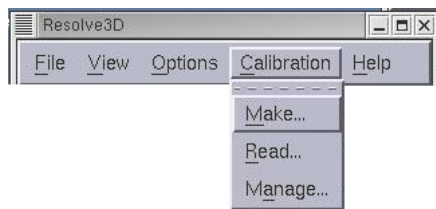
Save Settings

Saves the configuration settings and information (current filters, image size, etc.) to be used the next time Resolve3D is opened.

The Resolve3D Calibration Menu

Use the Calibration menu to make calibration tables, read calibration tables, and designate which calibration tools are active.

Figure E-5: The Resolve3D Calibration Menu



Make

Opens the Calibration tool that is used to create flat-field calibration tables and optional tables of "bad" pixels. These tables may be applied to images when they are acquired or at a later time. (To apply the tables to images after they are acquired, use the Calibrate utility available from the Process item on the main *softWoRx* menu.)

Read

Opens the Read Calibration Files dialog box that you can use to read calibration tables.

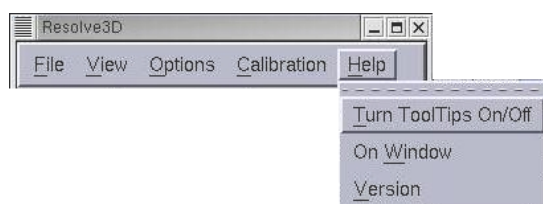
Manage

Opens the Manage tool that you can use to designate which calibration tables are active and to remove tables from the system's session memory.

The Resolve3D Help Menu

Use this menu to turn ToolTips on or off, get help on the Resolve3D window, or find out which version of *softWoRx* you are using.

Figure E-6: The Resolve3D Help Menu



Turn ToolTips On/Off

Turns tool tips on or off. Tool tips display “pop-up” information about buttons on the interface. They open when the mouse pointer is held over a button for a few seconds.

On Window

Opens Help for the Resolve3D window.

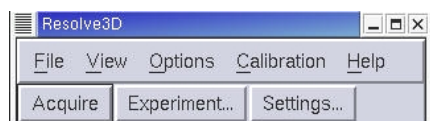
Version

Displays version numbers for the *softWoRx* components.

The Resolve3D Toolbar

Use the buttons on the Resolve3D toolbar to acquire images, open the Experiment Designer/Run dialog box that allows you to set up and run experiments, and open the Display Settings dialog box that allows you to control display, imaging, and file output options.

Figure E-7: The Resolve3D Toolbar



Acquire

Collects and displays an image from the microscope with the current settings. This image is only displayed in the Data Collection window. It is not saved in a file.

Experiment

Opens the Design/Run Experiment dialog box that you can use to select a previously created experiment, open the Experiment Designer window to design a new experiment, or open the Experiment Macro Editor to create or edit an experiment macro.

Settings

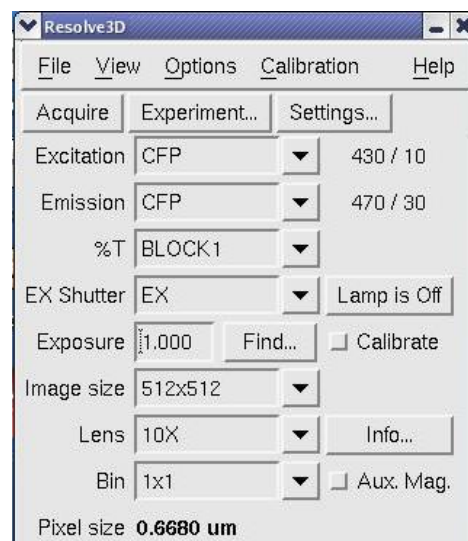
Opens the Settings dialog box that allows you to control display, imaging, and file output options.

Image Control Fields

Use the Resolve3D Image Control fields to:

- Select the Excitation, Emission, and Neutral Density filters.
- Select exposure time.
- Determine whether to calibrate the image.
- Select the desired shutter.
- Select the image size.
- Select the lens and get lens information.
- Select whether to use auxiliary magnification.
- Determine binning parameters.
- Get pixel size.

Figure E-8: The Resolve3D Image Control Fields



Excitation

Specifies an excitation filter. When this filter is selected, a corresponding emission filter is automatically selected and the following parameters are set to the last values that were used for that excitation filter.

- Exposure time
- Neutral Density filter
- Active illumination shutters
- Number of frames to average
- Target intensity
- Camera gain

You can choose **Save Settings** from the Options menu to store the configuration. The wavelength/bandwidth of the selected filter is indicated to the right of the filter choice box.

Emission

Specifies an emission filter. The wavelength/bandwidth of the selected filter is indicated to the right of the filter choice box.

%T

Specifies a Neutral Density filter. The relative illumination intensity is indicated in the menu. (This value indicates the amount of light passing through the filter. A value of 100% indicates the light is unfiltered.)

Exposure

Specifies camera exposure time (in seconds). The minimum and maximum exposure times allowed for this field depend upon the camera type.

Find

Finds the exposure time necessary to reach the target intensity that is entered in the Target Intensity dialog box. (This dialog box opens when the Find button is clicked.) For dim samples, 300-900 counts are acceptable. For bright samples, 3000-3600 counts provide a high dynamic range without saturation.

Calibrate

Calibrates images when they are collected from the camera. If you use this option, you must load and activate a calibration table that fits the current imaging parameters (wavelength, image size, bin choice) before you acquire images.

Shutter Control

Selects the desired excitation shutter.

Image Size

Specifies image size (pixels or CCD detector elements) for acquired images. The pull-down list contains predefined sizes for convenience. Special sizes may be entered by choosing **Other...** from the pull-down list. (Image size must be a multiple of four.)

Lens

Specifies the objective lens name. The pull-down list contains the lenses that are known to be part of the microscope system.

Info

Opens the Lens Information dialog box that displays information about the current objective lens.

Bin

Specifies the number of CCD detector elements to add together to form one image element. Binning is applied in both the X and Y directions. It increases intensity but it decreases resolution. Pixel size is a function of binning.

Aux. Mag.

Specifies to use the microscope's 1.6x manual auxiliary magnification. (On IX70 stands, the manual auxiliary magnification is 1.5x. On IX71 stands that have a Cascade II camera, the auxiliary magnification is 2.0x)

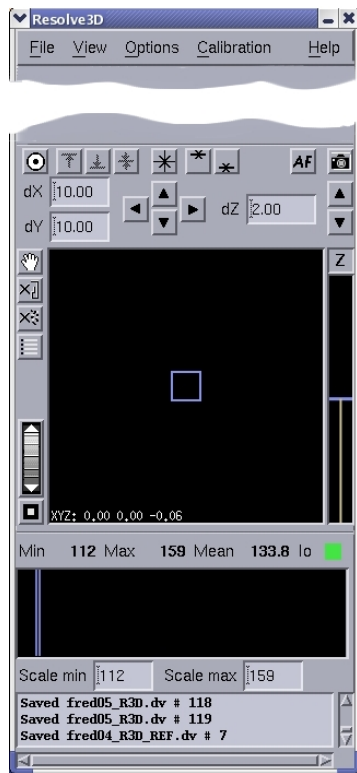
Pixel size

Displays pixel dimensions in microns/pixel. The value is calculated from the **Lens**, **Bin**, and **Auxiliary** magnification settings.

Stage Position Control Fields and Buttons

The Resolve3D Stage Position Control area allows you to control the microscope's X, Y, and Z stage positions. The Center, Mark, and Visit tools and the Stage Motion Controls are shown below.

Figure E-9: The Resolve3D Stage Position Control



Stage Control and Display Tools



Center Object

Centers the stage on an object selected in an Image Window. The pixel size must be correct in order for object centering to work properly, which means that the correct lens and auxiliary magnification setting must be selected. An image will be acquired after the object is centered.




Mark Point

Marks the current X, Y, and Z stage coordinates as points to be visited later.



Mark Top of Sample

Marks the current stage Z position as the "top" of your sample. Because the scanning process always moves the stage toward the objective, this position also represents the point where the stage is closest to the objective (the most negative Z value). This also represents the focal plane that is the closest to the slide side of a sample. Use this along with the Mark Bottom of Sample button to establish the thickness of the sample. You can use these marked positions to aid with the Z sectioning setup.

 **Note** All scans that are set up using the Experiment Designer scan in Z, using *relative* coordinates. The Mark Top and Mark Bottom buttons are most helpful in determining the thickness of the sample to be scanned. When an experiment is started, the scan region is determined by the current focus point and the thickness of the sample. So, for example, if you have marked three points to visit in an experiment and they all have different middle-Z positions, the experiment will calculate the scan based on these different Z positions and the fixed thickness.

**Visit Top**

Moves the stage to the position marked as the top of the sample.

**Mark Bottom of Sample (end of scan)**

Marks the current stage Z position as the "bottom" of your sample for a potential scan. This corresponds to the positive stage Z coordinate value or the coverslip side of the sample.

**Visit Bottom**

Moves the stage to the position marked as the bottom of the sample.

**Visit Middle**

Visits the point in the middle of the defined scan region.

**AutoFocus**

Automatically focuses.

**Acquire Image**

Collects and displays an image from the microscope. The current settings are used to collect the image. This image is only displayed in the Data Collection window. It is not saved in a disk file for later use.

**XY Stage Controls**

Moves the stage in the X and Y axis. The left and right arrows move the stage in the X axis in the increment set in the **dX** field and the up and down arrows move it in the Y direction in the increment set in the **dY** field.

**Z stage Motion Controls**

Moves the stage in the Z axis in the increment set in the **dZ** field.

**Pan**

Moves the stage view. This tool is "sticky." To disable it, click the Pan tool again.

**Clear Stage Trails**

Clears all of the stage trail lines from the stage view.

**Clear Thumbnails**

Clears all of the thumbnail images currently displayed on the stage view.



Marked Points list

Opens the Point List dialog box to manage the list of marked points.



Zoom Tool

Controls zoom. Drag the thumbwheel down to zoom in; up to zoom out. The button under the thumbwheel returns the zoom to 1:1.



Z Slider

Moves the stage up or down.

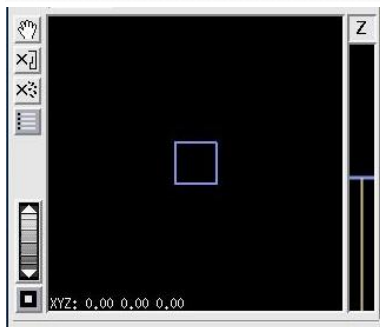
dX

Specifies the X step size, in microns.

dY

Specifies the Y step size, in microns.

Stage Trails Window



The blue box represents the current stage location. Drag the box to move the stage in X and Y.

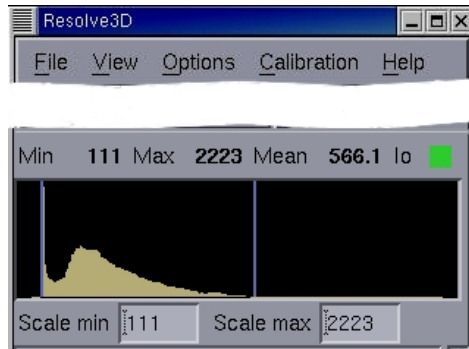
dZ

Specifies the Z step size, in microns.

Image Intensity and Scale Values

The Resolve3D Intensity values show the statistics for the intensity values of the image in numerical and graphical formats. The scale of the image is also shown.

Figure E-10: The Resolve3D Image and Intensity and Scale Values



Min, Max, Mean

Displays the minimum, maximum, and mean intensity values of the most recently acquired image. For a 12 bit CCD camera, these values range between 0 and 4095. A value of 4095 indicates camera saturation, unless image calibration is in effect. (If you are using 0.5X Gain, the saturation is less than 4095 counts.)

Histogram

Shows the intensity distribution for the most recently acquired image. The vertical blue bars indicate the **Scale min** and **Scale max** and can be dragged interactively with the mouse to change how the display is scaled. The X-axis represents intensity and the Y-axis represents the number of pixels.

Scale min/Scale max

Specifies the settings for the minimum and maximum display values. These numbers can be changed manually or by moving the histogram threshold bars.

lo

Indicates valid (or invalid) photo sensor values or saturation. The indicator is green if the photo sensor value is valid. It is red before the first image is acquired. After the first scan, a red color indicates that the most recently acquired image has an invalid photo sensor value. A red indicator can also signify unreasonable saturation or an improperly functioning photo sensor device.

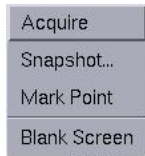
The Message Pane

The Resolve3D Message pane reports the status of various Resolve3D activities. Use the scroll-bar to view messages that have scrolled off the top of the pane.

Resolve3D Shortcuts

You can right-click anywhere in the Resolve3D window to open a shortcut menu that allows you to acquire images, mark points, and create a blank screen (for imaging under very light-sensitive conditions).

Figure E-11: Resolve3D Shortcut Menu



Acquire

Collects and displays an image from the microscope. The current settings are used to collect the image. This image is only displayed in the Data Collection window. It is not saved in a disk file for later use.

Snapshot

Launches a tool to let you collect a single 2-D, multi-wavelength "Snapshot" image. (The current settings are used to collect the image.)

Mark point

Marks the current X, Y, and Z stage coordinates as a point to be visited later.

Blank Screen

Turns the computer screen black for imaging under very light-sensitive conditions. Clicking anywhere on the screen restores it.

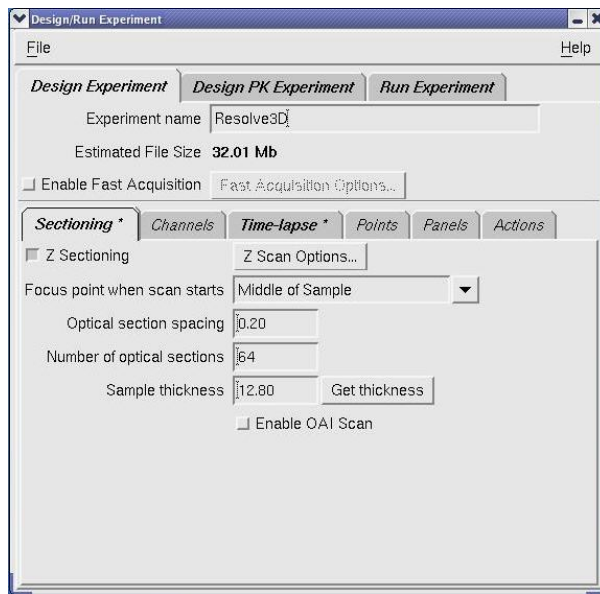
The Design Experiment Tab

The Design Experiment tab is used to generate experiment command macros.

To open the Design Experiment tab:

- From the Resolve3D window choose **Experiment**. The Design/Run Experiment window opens with the **Design Experiment** tab selected.

Figure E-12: Design Experiment

**Experiment name**

Specifies the name of the experiment macro.

Enable Fast Acquisition

Enables fast acquisition experiments (see Page 198).

Sectioning

Specifies sectioning for 3D images (see Page 198).

Channels

Specifies channels (filters) and exposure time (see Page 200).

Time-lapse

Specifies criteria for time-lapse experiments (see Page 201).

Point Visiting

Specifies a list of marked points (see Page 202).

Panels

Allows you to set up panel collections that you can use to review a large area of a slide or to stitch together to form a single, large image (see Page 203).

Actions

Lets you designate laser events, Autofocus, pause, wait, time-lapse, ratio imaging, and other specific actions to occur during the experiment.

Experiment name and Enable Fast Acquisition

Experiment name

Specifies the name of the file that is generated by the Experiment Designer. A file extension of .exp will be added to the name.


Enable Fast Acquisition

Enables fast image acquisition of 2D images. (This is only available when you are using an interline camera.)

This data acquisition mode should be used carefully. Fast Acquisition uses a single command to set up a data stream to the Instrument Controller. Because no checking for user input occurs once the command is executed, you cannot stop an acquisition sequence once it is started.

There is an upper memory limit for data collection that is based on the size of RAM memory for the Instrument Controller (typically about 350-400 MB). If you run into this limitation, turning Fast Acquisition off allows you to collect larger data sets.

If the system is unstable after collecting a large data set with Fast Acquisition, restart the workstation or stop using Fast Acquisition unless it is strictly necessary. Sometimes Fast Acquisition isn't much faster than standard acquisition, and more is risked than gained by using it.

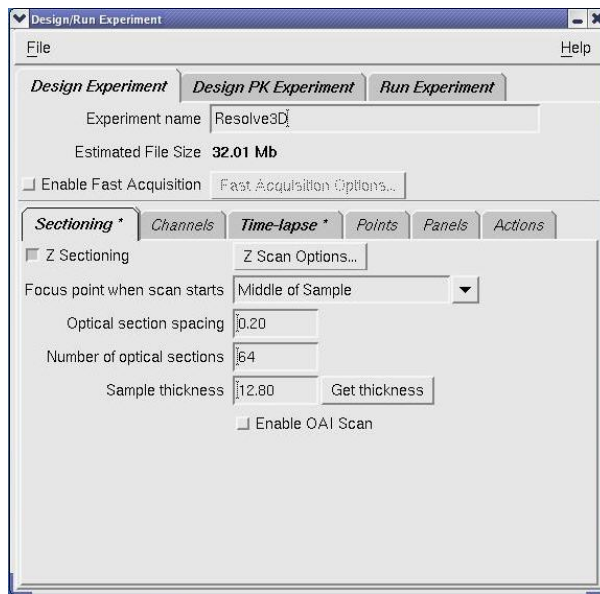
 **Note** You can set Fast Image Acquisition options to control the scan sequence, the shutter open mode, the camera readout mode, and the starting Z location for the scan. (See the online Help for more information.)

Sectioning Setup

Once your microscope is focused near the middle of the vertical zone of interest of your sample, you can use the parameters in the Design Experiment Sectioning tab to control the Optical Sectioning procedure.

 **Note** The standard scan direction moves the objective lens towards the specimen.

Figure E-13: Design Experiment Sectioning Setup Options



Focus point when scan starts

Specifies the Z location of the sample when the experiment starts. The recommended option is **Middle of Sample**.

Optical section spacing

Specifies the spacing (in microns) between each optical section. The focal point will be changed by this value after each image is collected.



Number of optical sections

Specifies the number of sections to collect (for each wavelength) for the experiment. *softWoRx* automatically calculates and displays this value if the **Sample thickness** and **Optical section spacing** are entered. If you specify this value, the **Sample thickness** value is changed.

Sample thickness

Displays the **Number of optical sections** multiplied by the **Optical section spacing**.

Get Thickness

Retrieves the Sample thickness, based on the locations defined with the  and  buttons in the Resolve3D window.

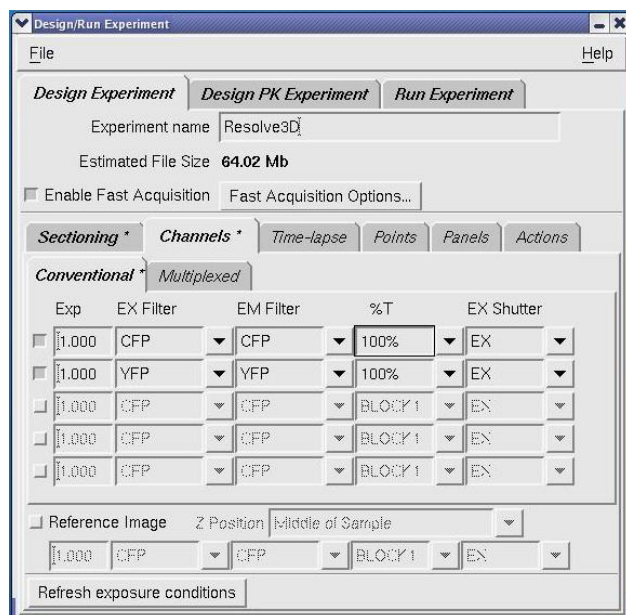
OAI

Acquires a 3D Z projection of the interval defined by the marked top and bottom of the sample. The optical section spacing and the number of optical sections are determined automatically, based on the depth-of-field of the objective and the exposure time.

Channels Setup

Use the Design Experiment Channels tab to select wavelengths (filters) and to specify an exposure time for each filter.

Figure E-14: Design Experiment Channels Options



Refresh exposure conditions

Updates the filter and exposure settings to those last used in the main Resolve3D window.

Active Wavelength Toggle Buttons

Enable the exposure time, filters, and display settings for specific wavelengths. Select the buttons that activate the wavelengths that you want to collect. (You must select one button for each wavelength.) If no wavelengths are selected, the exposure and filters that are set in the Resolve3D window are used.

Exp

Specifies the exposure time (in seconds) to be used when acquiring an image for the selected wavelength. If left blank, the value specified in Resolve3D will be used when the experiment is run.

EX Filter

Specifies the excitation filter to use for this experiment or image. When it is changed, the currently "paired" emission filter is automatically selected.

EM Filter

Specifies the emission filter to use. This filter may be selected independently of the EX Filter setting.

%T Filter

Specifies the Neutral Density filter to use. The % value indicates light transmittance. A value of 100 % indicates that no light is blocked (or no filtering).

Ex Shutter

Specifies the excitation (illumination) shutter to use for each channel of the image.

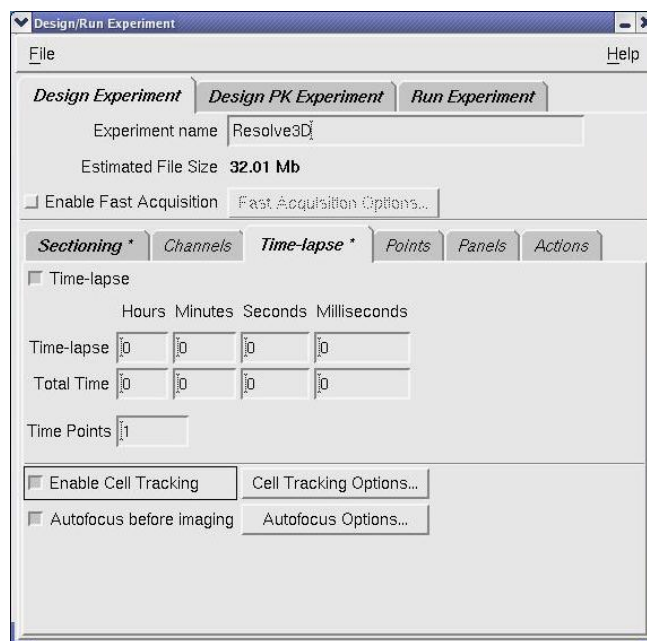
Reference Image

Specifies to use an alternate filter or the transmitted light to acquire a reference image that can be combined with other images. This option is useful for Differential Interference Contrast (DIC) analysis. It can also be useful for other types of reference images. Only one reference image per Z stack is collected.

Time-lapse Setup

Use the Design Experiment Time-lapse tab to specify the number of time points, the time periods, and the total time for a time-lapse experiment.

Figure E-15: Experiment Designer Time-lapse Setup Options

**Time-lapse option**

Specifies running a time-lapse experiment.

Time-lapse

Specifies the hours, minutes, and seconds for each time period.

Total Time

Displays the total time of the experiment, which can also be calculated as follows:

$$\text{Total time} = (\text{The number of time points} - 1) \times \text{time-lapse}$$

Time Points

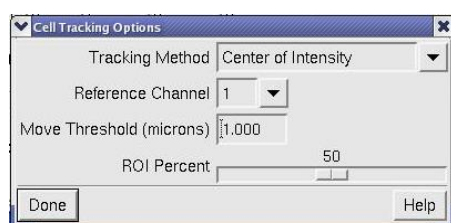
Specifies the number of time samples to collect in a time-lapse experiment.

Enable Cell Tracking

Moves the stage laterally to follow cells as they move during a time-lapse experiment. With the **Enable Cell Tracking** option selected, *DeltaVision* automatically keeps cells in the field of view.

Cell Tracking Options

Opens the Cell Tracking Options dialog box that allows you to set the parameters for cell tracking. For more information on using this dialog box, see Tracking Cells on Page 63.



Autofocus before imaging

Automatically focus the camera before each time point. For point visiting experiments, the camera is automatically focused every time that a point is visited.

Point Visiting Setup

Use the Design Experiment Point Visiting tab to specify a list of marked points to visit during the experiment.


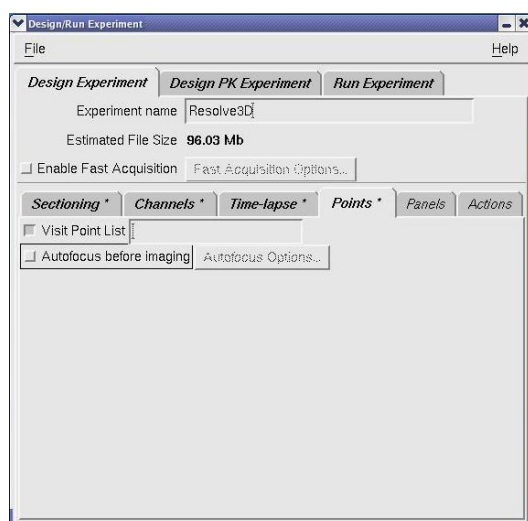
 **Tip** Before you specify these points, make sure that the point list is open in the Point List dialog. You cannot specify the points when the point list is closed.

Figure E-16: Experiment Designer Point Visiting Setup Options



Visit Point List

Specifies the list of points, described by number, to visit during the experiment. All sectioning and wavelength procedures are repeated at each of the listed points. A point list can be entered as a series of numbers separated by commas or dashes. Separating two numbers with a dash '-' (as shown in the following example) indicates that all point numbers in between should also be visited.

For example:

1, 2, 5, 7-10

Note When you use Point Visiting with Z sectioning, the microscope uses the Z value of the current focus of each point as the initial reference for that point. Values specified for Z sectioning are incremented relative to that point.

Autofocus before imaging

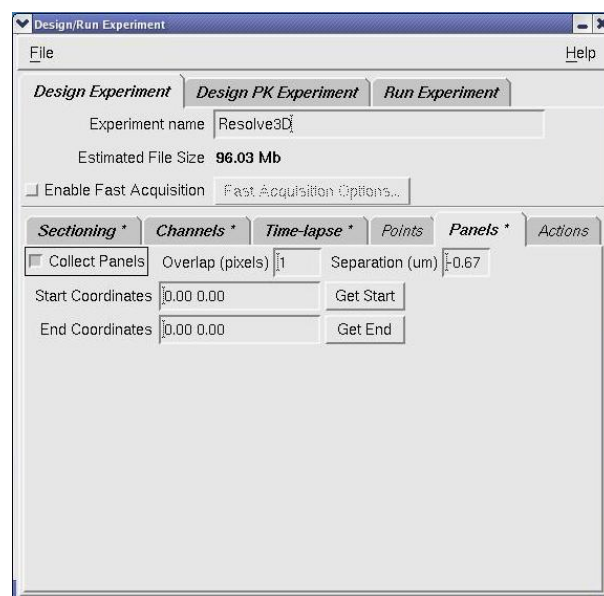
Automatically focus the camera at each visited point before acquiring an image.

Panel Collection Setup

Use the Design Experiment Panels tab to create panel collection macros. These macros are useful when you want to scan a large area with a relatively high magnification lens. You can use the panels as a means of reviewing a large area of a slide, or as data that you want to stitch together to form a single, large image.

Panel collection macros are sensitive to microscope settings such as image size and magnification. Because the number of panels required depends upon many factors, it is usually not a good idea to reuse panel collection macros.

Figure E-17: Experiment Designer Panel Collection Setup Options



Collect Panels

Specifies to use a panel collection macro.

Overlap (pixels)

Specifies the amount of overlap (in pixels) between adjacent panels.

Start Coordinates

Specifies the XYZ coordinates at which to start collecting panels. Use the **Get Start** button to obtain the current XYZ stage coordinates.

End Coordinates

Specifies the XYZ coordinates at which to finish collecting panels. Use the **Get End** button to obtain the current XYZ stage coordinates.

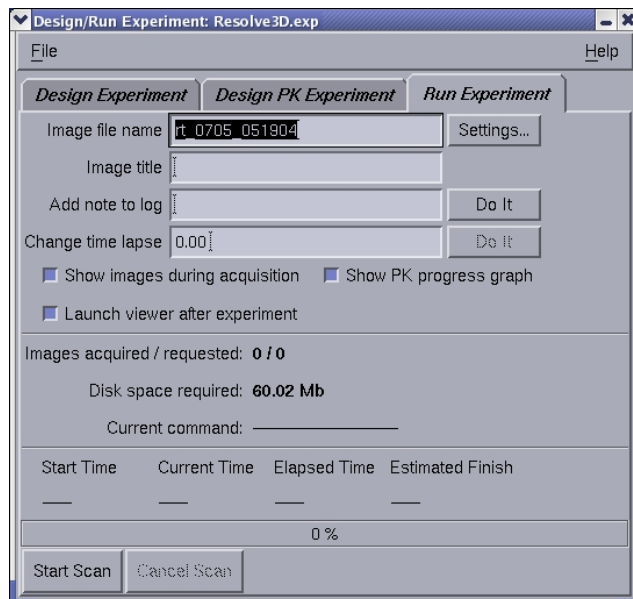
The Design/Run Experiment Dialog Box

The Design/Run Experiment dialog box provides tools to select, design, edit, and execute experiment macros. (Experiment macros are "scripts" of commands that guide the *DeltaVision* system to collect images.)

To open the Design/Run Experiment dialog box:

- From the Resolve3D window, click **Experiment** and click the **Run Experiment** tab.

Figure E-18: The Design/Run Experiment dialog box



Note The Design PK Experiment tab is available only for systems that have the QLM module.

Image file name

Specifies the file name to use for the image in this text field. If you do not provide a file name, you will be asked to provide one when the experiment starts running. If you have the **Auto-increment file** names setting (in the Settings dialog box) turned on, you will only need to provide a name once for each session. The names will have incrementing numbers appended to them automatically.

Image title

Specifies text to save in the header of the image file created by the experiment. The title can be viewed later using the **Header Labels** button of the Image Window's Image Information dialog box.

Add note to log

Inserts a note in the experiment's log file at any time before or while an experiment is running. (You will need to click **Do It** to insert the note.)

Change time lapse

Changes the time lapse value while an experiment is executing. (You will need to type the desired time, in seconds, in the text field and click the **Do It** button to change the value.) This value only affects experiment macros that contain a TLAPSE command. It does not permanently change the macro.

Show PK Progress graph

See the QLM Getting Started Guide.

Show images during acquisition

Displays images in a Data Collection window as they are collected. In cases where acquisition speed is important, deselecting this option may increase performance.

Launch viewer after experiment

Launches an Image window when the experiment is finished. Note that if more than two files are created in a point-visiting experiment, the Image windows will not launch even if this option is selected.

Images acquired/requested:

Displays the current number of images acquired, compared to the total number requested. (During fast acquisition, the reported number of acquired images may not be updated regularly.)

Disk space required

Displays the estimated size of the experiment (i.e., the size of the image file). Resolve3D checks the disk space to make sure that enough space is available before it runs the experiment.

Elapsed time:

Displays the elapsed time of the experiment, in seconds.

Estimated Finish

Displays the estimated clock time in which a running time-lapse experiment will finish.

Current command:

Reports each macro command as it is executed.

Start Scan

Starts the selected macro to run an experiment.

Cancel Scan

Terminates a scan while the experiment is running. (The images that are collected before the experiment is cancelled are automatically saved.)

Help

Opens the Help for the Design/Run Experiment dialog box.

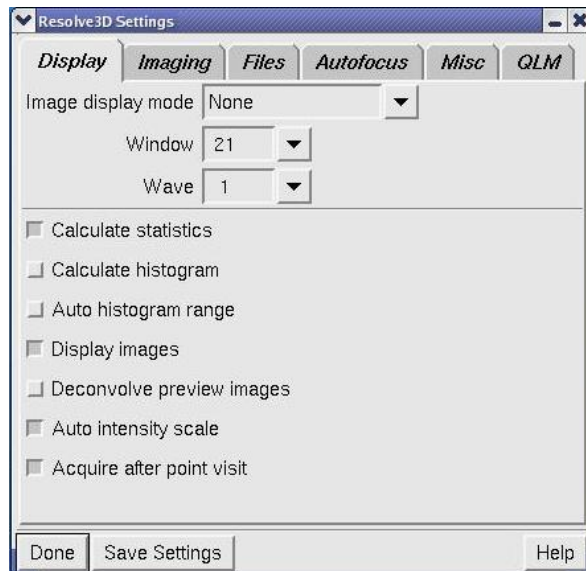
The Settings Dialog Box

Use the Resolve3D Settings dialog box to control how images are displayed, select camera settings, and specify file output.

To open the Settings dialog box:

- From the Resolve3D Window, click the **Settings** button.

Figure E-19: The Settings Dialog Box Display Options



Note The QLM tab is available only for systems that have the QLM module.

Settings Dialog Box Display Options

The Settings dialog box Display tab options allow you to control how the images are displayed.

Image Display Mode

Specifies which window and wave are used for image display. Five modes are currently available:

Model	Description
None	Displays images in the current window and wave.
Scratch	Displays all images in the specified window, Wave 1.
Auto Grayscale	Displays images in a separate window for each Emission (EM) filter.
Auto Color	Displays images in a different wave for each Emission (EM) filter. This option should be used only when you are running an experiment.
Point Track	Opens a separate window for each visited point in a point-visiting experiment.

Window

Specifies the number of the active display window. Select a new window to change where the next image will be displayed. (Temporary windows are numbered 21 or higher.)

Wave

Specifies the number of the active display wave (channel) as it is listed on the left side of the Data Collection window. Select a new wave to change which display window channel to use.

Calculate statistics

Calculates image intensity statistics. The typical reason for disabling this feature is to improve readout speed.

Calculate histogram

Calculates and displays the image intensity histogram in the Resolve3D window during image collection. (This option does not set scaling.)

Auto histogram range

Provides automatic scaling of the histogram width for each image that is analyzed. This option provides automatic histogram ranging from the minimum to the maximum intensity. When off, the histogram range remains at its current settings.

Display images

Displays images when the **Acquire** button is clicked or an experiment is running. Deselecting this option can increase performance.

Deconvolve preview images

Displays instantly processed 2D image previews that closely resemble images processed with advanced 3D image restoration techniques.

Auto intensity scale

Provides automatic scaling of the image intensity between the minimum and maximum brightness. (This switch applies only to the appearance of the image, not the actual data.)

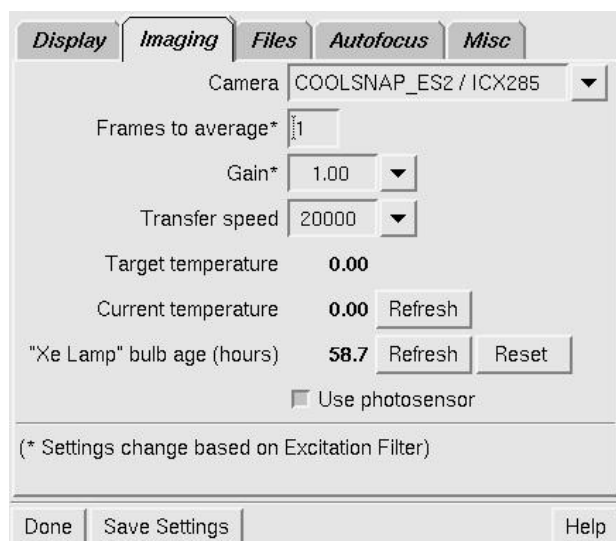
Acquire after point visit

Automatically acquires an image when the Visit Point option is selected.

Settings Dialog Box Imaging Options

The Settings dialog box Imaging options allow you to select camera settings.

Figure E-20: The Settings Dialog Box Imaging Options



Camera

Specifies which camera to use. For the EM CCD camera, this list also specifies whether to use Conventional or Electron Multiplication mode.

Frames to average

Specifies the number of successive camera images to average into a result that is displayed in the window. (This is similar to the AVG macro command.)

Gain

Specifies a gain value for the selected camera.

Transfer speed

Specifies a transfer rate for the selected camera (in kHz). Higher speeds may boost performance at the expense of image noise.

Target temperature

Displays the cooled CCD camera's target temperature. This can be changed only by modifying the appropriate configuration file on the Instrument Controller computer.

Current temperature

Displays the current temperature. Resolve3D monitors the temperature and updates this value occasionally. If you want to force a request for the current temperature, click the **Refresh** button.

Xe Lamp bulb age (hours)

Displays a count for the current number of hours on the xenon bulb.

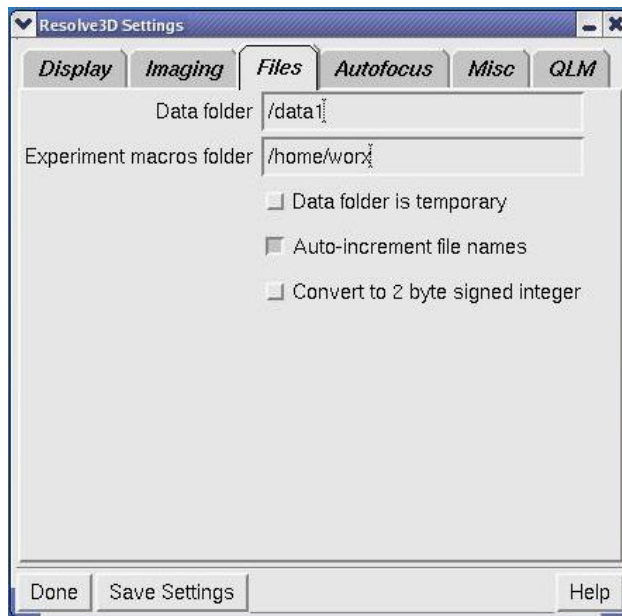
Use photosensor

Enables use of the Photo sensor.

Settings Dialog Box Files Options

Use the Settings dialog box File options to set the image output directory, set the directory where experiment macros are stored, and select auto incrementing for file names.

Figure E-21: The Settings Dialog Box Files Options



Data Folder

Specifies the destination directory for Resolve3D output images. If you supply a directory that you do not have permissions to use, you will be warned. If you provide a directory name that does not exist, you will be presented with the option of creating the directory. (You can also change the destination directory by changing the global *softWoRx* Image Data Directory in the User Parameters tool.)

Experiment macros folder

Specifies the directory where your experiment macros are stored.

Data folder is temporary

Specifies that any directory entered in the **Data Folder** field only applies to the current Resolve3D session. In subsequent Resolve3D sessions, the Data folder

value reverts back to the global *softWoRx* Data Directory as specified in User Parameters.

Use this option in environments where several users are running the microscope and using a single system login. Data folders can be created for each user under a common parent folder. As users start Resolve3D, they can choose to use their own folders.

In most cases, you should not use this option if each user has their own system login in your environment. With the option deselected, the Data folder assignment modifies the global *softWoRx* Data Directory definition and the system remembers the directory used in the last Resolve3D session.

Auto-increment file names

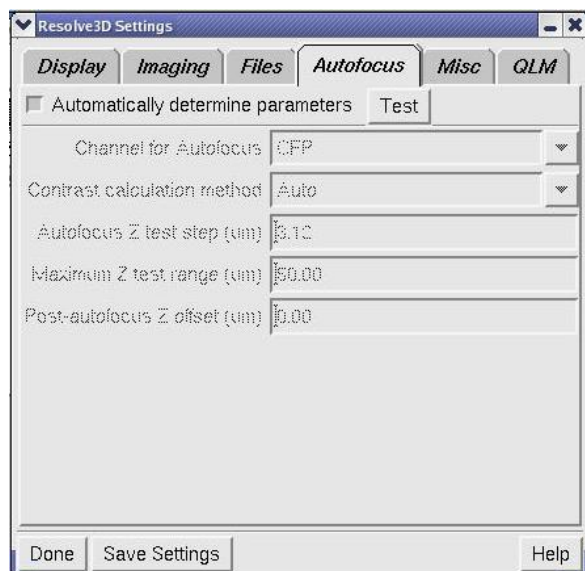
Creates a new file name by appending a serialized number to the base file name each time you run an experiment.

Convert to 2 byte signed integer

If toggled On, Resolve3D saves all images as signed 16-bit (the pre-3.5.0 default). This setting is designed to increase compatibility with older software. Note that this setting cannot be On for EMCCD cameras.

Settings Dialog Box Autofocus Options

Use the options on the Resolve3D Autofocus tab to set up Autofocus parameters.



Automatically determine parameters

Autofocus parameters are determined from the depth of field of the objective lens, which is calculated from the lens' numerical aperture. You must select the proper objective lens in order to get the appropriate Autofocus settings.

Channel for Autofocus

This setting indicates the wavelength to use for Autofocus.

Contrast calculation method

This setting determines the polarity of the contrast calculation. There are three choices for image contrast calculation methods:

- **Auto** – The instrument controller usually can determine which contrast calculation method to use, but not always.
- **Brightfield** – for dark objects on a light background
- **Fluorescence** – for light objects on a dark background

Autofocus Z test step (μm)

This option sets the step size used for Autofocus within the maximum Z range.

Maximum Z test range (μm)

The setting indicates the maximum range that the Autofocus will search.

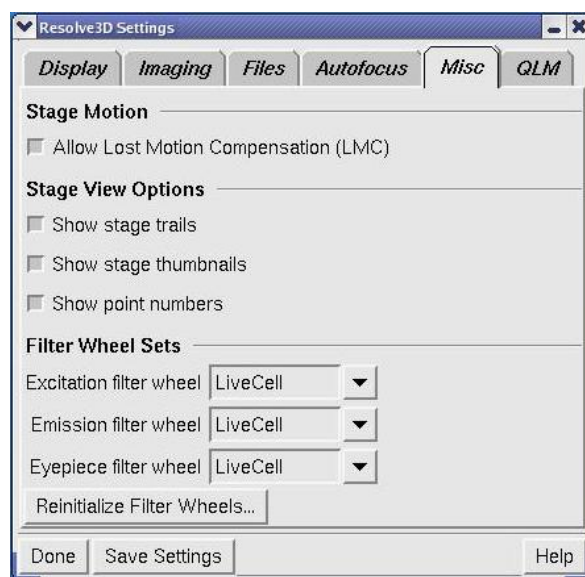
Post-autofocus Z offset (μm)

This setting can provide a constant offset after the Autofocus position determines the best plane of focus. Often times Autofocus will find a plane that is consistently different from the desired plane.

Settings Dialog Box Misc Options

Use the options on the Resolve3D Misc tab to select or clear Lost Motion Compensation or to select a filter wheel configuration (if your system is configured to use alternate filter wheels).

Figure E-22: The Settings Dialog Box Misc Options



Allow Lost Motion Compensation (LMC)

Enable Lost Motion Compensation to remove the effect of hysteresis in the stage. This option should be selected for most applications. When it is turned off, the Z series range may shift. Clear this option if you want to improve speed at the expense of position repeatability.

Show stage trails

Display the path of stage movement on the Stage View.

Show stage thumbnails

Display a thumbnail image of each image on the Stage View as the image is acquired.

Show point numbers

Displays the number of each point in a point list on the Stage View.

Excitation filter wheel

Allows you to select the name of the Excitation filter wheel that you are using. This option list appears only if you have configured your system for alternate filter wheels.

Emission filter wheel

Allows you to select the name of the Emission filter wheel that you are using. This option list appears only if you have configured your system for alternate filter wheels.

Eyepiece filter wheel

Allows you to select the name of the Eyepiece filter wheel to use. This option list appears only if you have configured your system for alternate filter wheels.

Reinitialize Filter Wheels

Reinitializes the filter wheels.

Action Buttons

Done

Closes the Resolve3D Settings dialog box.

Save Settings

Preserves the current options for your next Resolve3D session. In addition to options in the Resolve3D Settings dialog box, current state information such as current filters and exposure time is saved.

Help

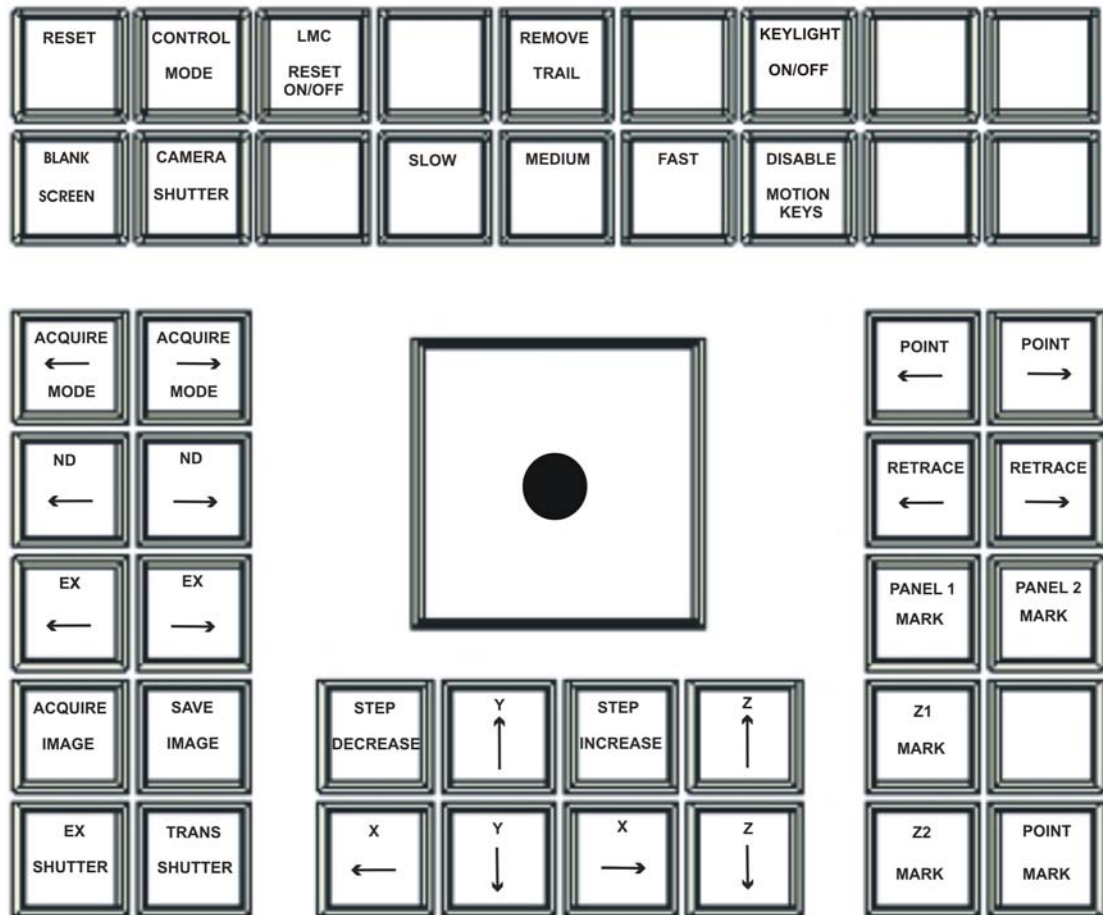
Opens the Help for the Settings dialog box.

Keypad/Joystick Operation

Many of the functions accessible through Resolve3D are also available on the keypad/joystick. This section describes each key on the keypad/joystick.

 **Note** Some buttons on your keypad may not be active.

Figure E-23: Keypad/Joystick



RESET


Clears communication buffers, closes shutters, stops all motors, and clears encoder errors. Use the RESET button when you suspect that the workstation and controller are not synchronized.

CONTROL MODE

Toggles the controller mode between local mode and remote mode. Local mode enables control by the keypad and joystick. The remote mode disables the joystick and the keypad buttons (except for CONTROL MODE and a few other buttons) and shifts control to other internal components.

LMC RESET ON/OFF

Executes a Lost Motion Compensation (LMC) move.

 **Tip** You can disable LMC on the Resolve3D Settings dialog. (For more information, see the online Help.)

REMOVE TRAIL

Clears the stage movement history from memory.

KEYLIGHT ON/OFF

Turns the keypad backlight on and off.

QUIT

Quits the controller program when pressed twice. (To restart the Instrument Controller program, double-click the Instrument Controller icon on the desktop.)

BLANK SCREEN

Suspends (or activates) the monitor's light display (BLANK SCREEN is a toggle button). Use this feature when viewing dim samples or performing light sensitive experiments.

SLOW/MEDIUM/FAST

Adjusts the joystick stage movement speed.

DISABLE MOTION KEYS

Disables the eight keys below the joystick. This prevents accidental input from the keys when using the joystick.

YES/NO

Allows response to questions prompted on the screen. For example, during initialization, the computer will ask if you want to continue with initialization. The Yes and No buttons are an easy way to respond.

ACQUIRE MODE ARROWS

Changes the acquisition mode. (This includes the Excitation filter, exposure time, shutter configuration, and many of the options defined in the Settings dialog box.)

JOYSTICK

Moves the stage in X and Y.

POINT ARROWS

Scrolls through the list of marked points. Press ACQUIRE IMAGE to view the image for a selected marked point.

ND ARROWS

Changes the neutral density filter to one of a lower (←) or higher (→) value. When the neutral density filter is at the minimum value and you push the decrease arrow (←), the wheel moves to engage the maximum value filter. When the

Neutral Density filter is at the maximum value and you push the increase arrow (→), the wheel moves to engage the minimum value filter.

RETRACE ARROWS

Retrace (←) moves the stage one position back in the stage movement history buffer. Retrace (→) moves the stage one position forward in the stage movement history buffer.

EX ARROWS

Changes the excitation filter to the previous (←) or next (→) filter on the filter wheel. This button changes the excitation filter but not any other settings, such as exposure time and shutter configuration.

PANEL 1/2 MARK

Defines the area of the specimen to be stitched, as shown in the following diagram.

Figure E-24: Panels Diagram



ACQUIRE IMAGE

Commands the system to collect image data. This key function is identical to clicking **Acquire** in Resolve3D. Use this key when you are scanning through your sample and using the eyepiece to find a region of interest, or when you want to get a quick look at the specimen on the monitor.

SAVE IMAGE

Saves the last acquired image to the currently open file.

STEP DECREASE/INCREASE

Changes the step size of the movement controlled by the arrow keys located beneath the joystick. Pressing either button many times will change the step size to the minimum/maximum. There are 8 possible step sizes: 50nm, 100nm, 200nm, 400nm, 800nm, 2250nm, 4500nm, and 9000nm.

X AND Y ARROW KEYS

Move the stage in the direction shown.

Z ARROW KEYS

Move the stage in the direction shown.

Z1 MARK/Z2 MARK

Define the boundaries of the Z series.

EX SHUTTER

Toggles the excitation shutter between open and closed.

TRANS SHUTTER

Toggles the shutter for the transmitted light source between open and closed.

POINT MARK

Adds the stage position to the marked points list.

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